JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXVII WASHINGTON, D. C., JAN. 5, 1924 NO. 1

PHYSIOLOGICAL STUDIES ON APPLES IN STORAGE[1, 2]

By J. R. MAGNESS, *Physiologist, and* H. C. DIEHL, *Junior Physiologist, Office of Horticultural Investigations, Bureau of Plant Industry, United States Department of Agriculture*

The successful holding of apples in storage is of great importance in this country, not only from the point of view of the commercial fruit industry, but from the viewpoint of the consuming public as well. Fresh fruit is assuming a position of increasing importance in the diet of the human race. The marketing of the fall and winter varieties of apples direct from the tree is practically limited to three months of the year. If a supply of this fruit is to be available throughout the year, storage suitable for holding the fruit is essential. Not only does proper storage insure a supply of a necessary food material throughout the year, but it also greatly stimulates consumption and stabilizes prices for the fruit grower.

Although much study has been devoted to the question of proper storage conditions for apples and other fruit, the problems involved are so complex that many questions remain unanswered. The factors of the exact influence of time of picking, storage temperature, humidity, ventilation, etc., upon the physiological ripening processes in the fruit are not perfectly understood, and many inaccurate impressions exist in regard to their functions in storage management.

In the studies here reported, the literature concerning the chemistry and physiology of the ripening processes in apples have been freely drawn upon and correlated with the investigations on the storage life of the fruit made by the authors.

THE RIPENING OF APPLES ON THE TREE

For a proper understanding of the changes that occur in apples while in storage, it is important that the processes of growth and development of the fruit while on the tree be considered. The physiological changes in the apple fruit from the time of blossoming until ultimately consumed or until the end of its life is reached are continuous. The conditions under which the fruit is grown and the time of picking have an important bearing upon the storage behavior of the fruit.

PHYSICAL CHANGES DURING DEVELOPMENT OF THE FRUIT ON THE TREE

Among the most important of the physical changes in apples during development and ripening on the tree may be mentioned increase in size, changes in color, changes in the skin texture, and changes in hardness of the flesh. These changes are all intimately associated with storage quality in apples.

[1] Accepted for publication Nov. 19, 1923.

[2] This paper gives the results of a portion of the work carried on under the project "Factors Affecting the Storage Life of Fruits."

INCREASE IN SIZE OF THE FRUIT

The data on increase in size of apples, particularly during the latter part of the growing season, are extremely meager. Whitehouse (*27*) [3] measured the longitudinal and transverse diameters of Grimes apples at intervals, and found a steady increase in size from early June until September 20, when the last records were made. Actual diameter increase was somewhat less during the late season, but volume increase was very rapid. Curves based on Whitehouse's data are shown in Figure 1. In Figure 2 are shown measurements on diameters of 50 Winesap apples at Arlington, Va., during the summer of 1919.[4] Since the volume of the fruit increases approximately as the cube of the diameter, the second curve has been calculated to show the comparative increase in volume.

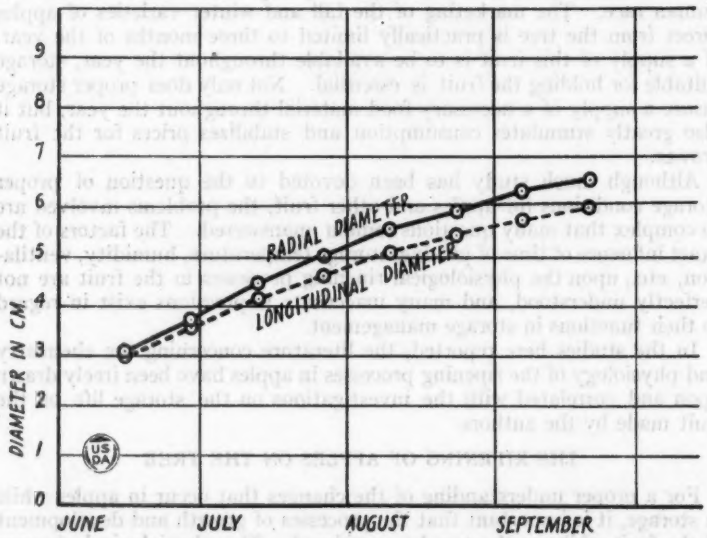


FIG. 1.—Increase in diameter of Grimes apples, Corvallis, Oreg., in 1915, after data by Whitehouse.

The season of 1919 at Arlington had a very heavy rainfall in July, somewhat less than normal in August, less than half of normal in September, and a heavy rain on October 1. It will be noted that there was a rapid increase in size from July until August 21, with only slow growth until September 19. A very rapid growth occurred between September 19 and October 10, most of which probably occurred after the heavy rain of October 1. No frost occurred in October.

These data indicate that if there is moisture available a large increase in size may occur very late in the growing season. There is much need of further investigations to determine how late this growth will continue under various conditions. Quantity of fruit on the tree as well as soil moisture will markedly influence the rate of increase in size. The size

[3] Reference is made by number (italic) to "Literature cited," p. 36–38.

[4] The authors are indebted to L. A. Hawkins, physiologist, and L. B. Scott, pomologist, Office of Horticultural Investigations, for these data.

that the fruit has attained will often be an important consideration in determining when the fruit should be picked.

Increase in size of the fruit, at least during the latter part of the growing season, appears to be due largely to increase in size of the individual cells rather than to increase in their number. Consequently, fruit which attains large size will be composed of larger cells, and will be less firm and compact in texture than small or medium sized apples of the same variety. Medium or small sized fruit with compact firm texture is almost invariably superior for storage purposes to large size fruits, but the

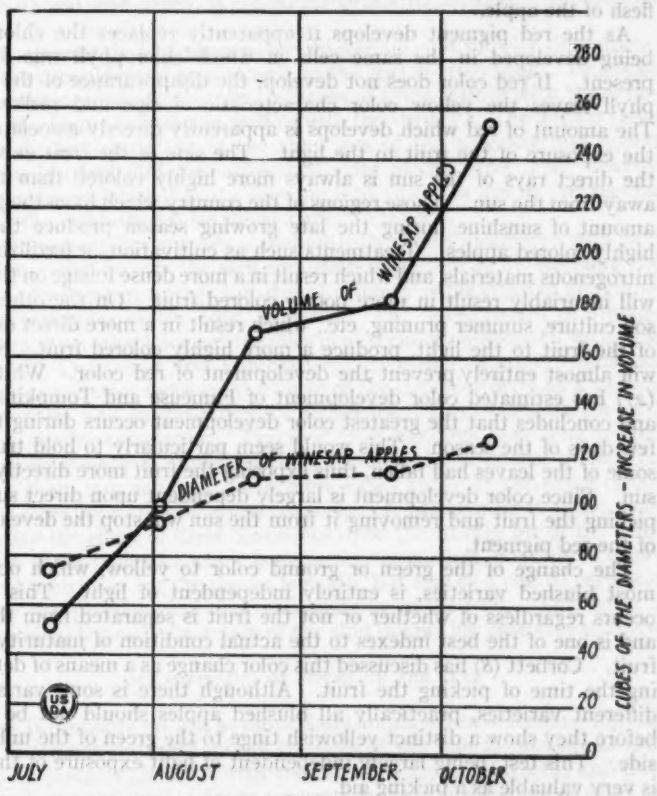


FIG. 2.—Increase in diameter and in volume of Winesap apples at Arlington, Va., 1919.

market usually pays a premiun for large size in apples. Cummings and Lombard (*9*) have pointed out an apparent association between size of cells and storage quality in apples.

CHANGES IN COLOR ASSOCIATED WITH RIPENING ON THE TREE

The changes in color as the fruit ripens on the tree are twofold. There is a gradual increase in the red pigment developed in the subepidermal cells, the color increasing both in area and in intensity as the season advances. At the same time, the green or ground color gradually gives way to greenish yellow and finally to yellow.

The chemical changes which are responsible for color development in the apple have not, so far as the writers are aware, been determined. The cells in which the pigment is located are primarily subepidermal. There is no pigment in the cuticle, or wax covering, and no color in the epidermis, or outer cell layer. Below the epidermis, however, chlorophyll pigment, and later the red pigment, is very abundant. These pigments are largely limited to the rather flat, thick-walled cells lying in the immediate 2 to 10 layers beneath the epidermis, though in certain varieties some pigment, both green and red, occurs well down into the flesh of the apple.

As the red pigment develops it apparently replaces the chlorophyll, being developed in the same cells in which chlorophyll was formerly present. If red color does not develop, the disappearance of the chlorophyll leaves the yellow color characteristic of ripe and mellow fruit. The amount of red which develops is apparently directly associated with the exposure of the fruit to the light. The side of the fruit exposed to the direct rays of the sun is always more highly colored than the side away from the sun. Those regions of the country which have the greatest amount of sunshine during the late growing season produce the most highly colored apples. Treatments such as cultivation, or fertilizing with nitrogenous materials, and which result in a more dense foliage on the trees, will invariably result in more poorly colored fruit. On the other hand, sod culture, summer pruning, etc., which result in a more direct exposure of the fruit to the light, produce a more highly colored fruit. Shading will almost entirely prevent the development of red color. Whitehouse (*27*) has estimated color development of Fameuse and Tompkins King, and concludes that the greatest color development occurs during the last few days of the season. This would seem particularly to hold true after some of the leaves had fallen, thus exposing the fruit more directly to the sun. Since color development is largely dependent upon direct sunlight, picking the fruit and removing it from the sun will stop the development of the red pigment.

The change of the green or ground color to yellow, which occurs in most blushed varieties, is entirely independent of light. This change occurs regardless of whether or not the fruit is separated from the tree, and is one of the best indexes to the actual condition of maturity of the fruit. Corbett (*8*) has discussed this color change as a means of determining the time of picking the fruit. Although there is some variation in different varieties, practically all blushed apples should not be picked before they show a distinct yellowish tinge to the green of the unblushed side. This test, being largely independent of light exposure of the fruit, is very valuable as a picking aid.

CHANGES IN THE SKIN OF APPLES DURING RIPENING

The changes in the skin of the apple during its development on the tree have been studied in detail by Zschokke (*28*). He found the skins of young apples 4 to 5 weeks old to contain 2 to 10 stomata per square millimeter. No further stomata were formed, and as the fruit increased in size the stomata became farther and farther dispersed. During the latter part of the growing season cork formation usually occurs below the stomata openings. The openings are later ruptured by the growth of the fruit. The corky tissue with the ruptured stomata constitute the "dots" by which many varieties are characterized. In some varieties, however,

Zschokke found the stomata ruptured before the cork cells had formed, in which case protection was provided by the drying down of the underlying tissue. This, however, afforded opportunity for the entrance of microorganisms.

The epidermal covering of the apple consists of a cuticle, or wax coating on the outside, a layer of thick-walled epidermal cells, and below this a subepidermal region, consisting of a number of layers of thick-walled small and compact cells. The cells of the subepidermal region gradually become larger as the distance from the epidermis increases. The pigment, both red and green, is contained primarily in these subepidermal cells.

There apparently is a progressive thickening, not only of the cuticle, but of the subepidermal area as well, as the season progresses. Zschokke found a distinct variation in the thickness of these regions with the exposure of the fruit to the sun as well as with the variety. The writers have noted a distinct variation in the thickness of the subepidermal region upon the blushed and the unblushed sides of the same fruit, heavily blushed sides of Rome Beauty showing 8 to 10 cell layers in this region, whereas the unblushed sides showed about 6 layers.

The storage quality of fruits is undoubtedly closely associated with skin condition. Not only is the skin condition important in determining the amount of wilting in storage but it is of primary importance in determining the resistance of the fruit to decay. The development of storage scald and other physiological troubles is also closely associated with skin condition. Cummings and Lombard (*9*), in a limited study, found an apparent association between thickness of the cuticular layer as a variety characteristic and keeping quality in apples. Perry and Martin (*23*) report a similar correlation. Practical fruit handlers have long judged the keeping quality of apples partially by the amount of wax present on them. There is much need for a more exact study of the effect of climatic factors upon the epidermal covering of fruits in order to judge more accurately the effect of these conditions upon the storage life of the fruit. The skin of the fruit as a whole is extremely important, not only as a mechanical protection for the tissues beneath, but also as a partial regulator of the actual physiological processes going on in the fruit. This latter is accomplished through the action of the skin in limiting gaseous exchange.

THE SOFTENING OF APPLES ON THE TREE

There is probably no test so universally employed to determine the maturity of fruit as the resistance of the flesh of the fruit to pressure as estimated by pressing the fruit with the thumb. Lewis, Murneek, and Cate (*18*) used a mechanical tester to measure the rate of softening of pears on the tree. They found the fruit to soften rather rapidly, resistance to pressure decreasing at a rate of approximately 2 per cent per day.

Apples are usually removed from the tree when in a "hard ripe" condition. Color, ease of separation from the tree, amount of dropping, color of seeds, etc., as well as the softness of the fruit, are used to determine the time of picking. Notwithstanding the fact that apples are usually picked when "hard ripe," there is in most varieties a very considerable softening of the fruit before its removal from the tree. The rate of this softening has been determined by means of a mechanical pressure tester similar in design to that described by Murneek (*21*).

Measuring the Softening

From 25 to 40 apples, selected to represent the averages of the trees from which they were taken, were used for each determination of the hardness of the fruit. Apples from the same trees were used in successive tests, made at intervals of from 5 to 7 days.

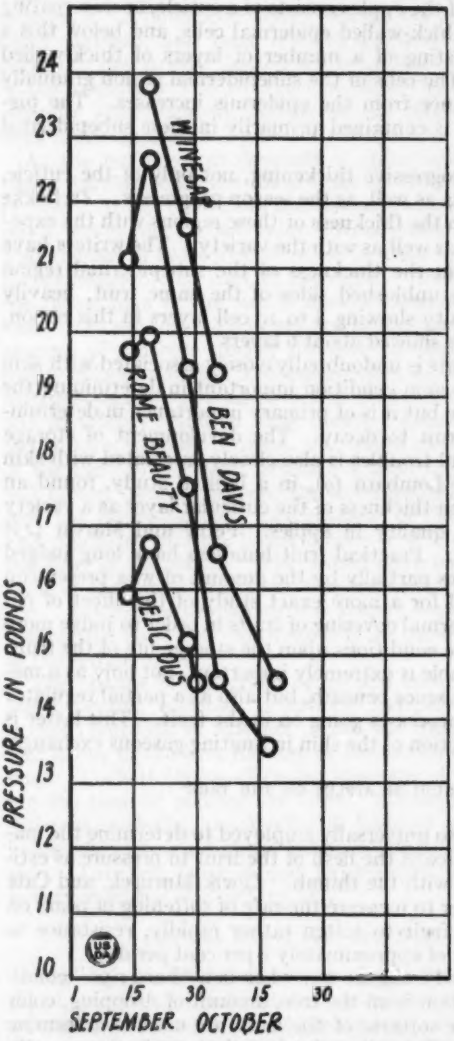


FIG. 3.—Softening of Winesap, Ben Davis, Rome Beauty, and Delicious apples while on the trees, Arlington, Va., 1922.

Three tests were made upon the exposed flesh of each apple. The skin was removed from an area of about three-fourths of an inch diameter at three points equidistant about the circumference of the fruit, and the test made of the number of pounds pressure required to force a smoothly rounded plunger seven-sixteenths of an inch in diameter into the flesh of the fruit for a distance of five-sixteenths of an inch. Tests were also made of the same apples with skin intact, but it has been found that tests directly on the flesh of the apples are a more accurate index to the real condition of the fruit.

Unfortunately, these tests were not begun until rather late in the season, when softening may have been considerably advanced. When initial tests were taken, however, the fruit was not well colored, and lacked very appreciably of having attained its final size. Exact growth measurements were not made.

Figure 3 shows in graphical form the resistance to pressure of Delicious, Rome Beauty, Winesap, and Ben Davis apples. At the time of making the initial pickings of Delicious, Rome Beauty, and Ben Davis the pressure-testing apparatus was not ready, and the fruit was held at 32° F. for

one week before testing. Initial tests on these varieties were lower than those following, probably because of this treatment. Under conditions prevailing during these tests, Ben Davis, Winesap, and Rome Beauty all softened very rapidly, and Delicious somewhat more slowly. The month of September, 1922, was very warm and dry at Arlington, Va., and October was practically without precipitation and moderately warm. Burroughs (*5*) found that Wagener apples growing on young trees in northern Pennsylvania softened only very slowly during September and October. These trees had received nitrate fertilizer and were very vigorous. It is not known whether the rate of softening while on the tree is primarily a variety characteristic or whether it varies largely with environmental factors.

In general, the harder the fruit is at time of picking and placing in storage the firmer it will be when removed from storage and exposed to market conditions. A firm, hard fruit, sufficiently well colored to be attractive, and sufficiently well ripened to be of high quality, and to escape storage scald, is the ideal for storage. High quality and skin condition associated with resistance to storage scald can be obtained only at the expense of a softening of the fruit. There is much need for a more exact knowledge of the influence of such factors as temperature, soil moisture, fertilizers, age and vigor of the trees, size of crop, etc., upon the rate of softening of the fruit. It is highly desirable that the fruit when picked be as firm as it is possible to secure it.

CHEMICAL CHANGES IN APPLES ASSOCIATED WITH RIPENING ON THE TREE

Numerous analyses have been made of the chemical composition of apples and other fruits at various times during their development on the tree. While the actual percentages of the various constituents will vary widely, depending upon the variety, locality, cultural conditions, and many other factors, the general changes have been well established and will be briefly reviewed. These changes may be considered under the following subjects: (1) Changes in total acidity, (2) changes in the sugar and starch content, (3) changes in the pectin content, and (4) changes in the moisture content of the fruit.

CHANGES IN TOTAL ACIDITY ASSOCIATED WITH RIPENING ON THE TREE

For more than a century, acidity changes in fruits have attracted wide attention, and many conflicting opinions have been held concerning the source of fruit acids, as well as their disappearance. This literature has been thoroughly reviewed by Bigelow, Gore, and Howard (*2*). Because of the limited chemical knowledge and the widely varying conditions under which early writers on this subject worked, many of their results are untrustworthy. Only the more recent contributions will be discussed here.

Browne (*4*) analyzed Baldwin apples and found a marked decrease in acidity at intervals from August 7 until December 15, when recorded as percentage of the wet weight of the apple. Gerber (*12*) reported a similar decrease in percentage composition, but pointed out the fact that the total amount of acid per apple increases rather than decreases as the season advances, the apparent decrease being more than counteracted by the increase in size of the fruit. Finally Bigelow, Gore, and Howard (*2*) in a very complete series of analyses on both summer and winter varieties of apples, found the percentage of acid to drop very rapidly from June until

September in all varieties. From the middle of September until the first of November the percentage of acid remained practically constant in the winter variety group which they analyzed (Winter Paradise, Huntsman, and Ben Davis). When the actual quantity of acid in grams per apple was calculated, however, these authors found that the amount of acid per apple increased until August, fell off very slightly during September, and again increased during October. It is probable that the decrease during September was due to local seasonal conditions.

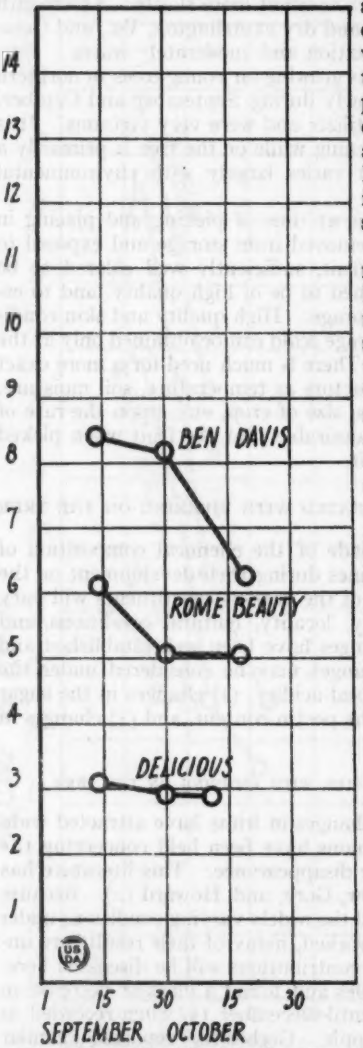


FIG. 4.—Decrease in acidity in apples while remaining attached to tree, Arlington, Va., 1922. Acid as cc. N/10 per 10 gm. wet tissue.

In Figure 4 the changes in acidity for Ben Davis, Delicious, and Rome Beauty grown at Arlington, Va., during the ripening season of 1922 are shown graphically. It is apparent that from September 14 until mid-October there was a constant, though rather slight, decrease in the total acidity on the basis of percentage composition. Unfortunately, data on the relative size of the fruit at the time different determinations were made are not available. The fact, however, that there was a marked increase in size, particularly in Rome Beauty and Ben Davis, between September 14 and October 18 strongly indicates that the actual acidity in grams per apple increased rather than decreased during this period.

To summarize, then, the changes in acidity occurring while the fruit is on the tree, there is a marked decrease in percentage of acid in the fruit during most of the growing season, this decrease becoming less pronounced as the fruit approaches maturity on the tree. This decrease in acidity does not involve an actual disappearance of acid from the fruit, as the increase in size of the fruit is sufficient to simply dilute the acid already there. The most accurate conception of the acidity changes in apples while on the trees apparently is that of high acidity early in the season, followed by dilution of the acid as the fruit increases in size, with consequent lower acid concentration in the mature fruit.

The amount of acid which the fruit of any variety may contain will vary widely with the conditions under which the fruit is grown. Con-

siderable variation occurs in individual apples from the same tree, depending upon the nutrition of the individual apples. Ballard, Magness, and Hawkins (*1*) have reported that apples from branches of Yellow Newtown trees which were girdled in June contained distinctly more acid, as well as more total sugar, than did apples from normal branches of the same trees, while fruit from partially defoliated branches on the same trees showed still less acid than the untreated checks.

In other works by the same investigators (unpublished) acidity determinations were made on fruit from a large number of Yellow Newtown trees during each of three succeeding seasons. These trees were about 20 years of age, and during the first and third seasons produced only a very light crop, averaging 1 to 6 bushels per tree. During the second season under test, however, the crop was very heavy. It was found that the average acidity in fruit from the same trees was fully 25 per cent higher during the "off" or light crop years than during the season of heavy production. These figures represent an average of about 70 individual trees. Furthermore, during the "off" year acidity was much lower on the average in the trees bearing a fairly full crop.

It is apparent, then, that there is a close association between acidity in apples and the nutritive conditions in the tree upon which they were produced. Climatic conditions also are of undoubted importance, although they have not been carefully studied. Acidity is of much importance in determining quality in fruit not only as relates to growing conditions, but as regards storage as well, for it is intimately associated with flavor.

CHANGES IN WATER CONTENT OF THE FRUIT

The change in water content of the fruit as it matures on the tree is less marked than changes in sugars. The percentage of water in the fruit will vary widely with variation in soil moisture. Conditions of soil moisture being uniform throughout the season, however, there is a slight decrease in the amount of water and an increase in dry matter as the season progresses. This change is not marked, but is borne out by the results obtained by Bigelow, Gore, and Howard (*2*), by Burroughs (*5*), and by other investigators.

CHANGES IN SUGARS AND STARCH DURING GROWTH

The literature regarding changes in sugar in fruit during its ripening on the tree is equally as voluminous as that regarding acidity changes. The following summary is based on the work of Bigelow, Gore, and Howard (*2*) which included a careful study of the sugar changes in apples both while attached to the tree and while held in storage following picking.

During early summer (June) the total sugar content of all the winter varieties was low, running from 3 to 4 per cent of the weight of the green tissue. In both summer and winter varieties, the percentage of total sugar increased steadily until the fruit was dropping badly, that being late July for the summer varieties (Early Strawberry, Bough, and Yellow Transparent) under West Virginia conditions, and late October for the winter varieties (Ben Davis, Huntsman, and Winter Paradise). During the early season, the sugar is largely in the form of reducing sugars, but as the season advances there is a marked increase in the

amount of sucrose present, as well as an increase in reducing sugar. In some varieties at the end of the growing season the quantity of reducing sugar and of sucrose was approximately equal, while in others the reducing sugar was approximately double the sucrose in quantity.

The starch content of the fruit, in terms of per cent of the green weight, reached its highest point in the summer varieties in June and in the winter varieties in July. The actual total quantity of the starch per apple, however, did not reach its maximum until much later, about mid August, in the winter varieties. From this time forward there was a decrease not only in percentage composition of starch but in actual starch per apple, indicating that there was an actual starch hydrolysis going on after this date. With starch hydrolysis there is a sharp increase in sucrose, indicating that the sugar storage form which replaces the starch storage as the fruit matures is primarily sucrose.

CHANGES IN THE PECTIN MATERIALS

There is much confusion regarding the changes that occur in the pectin materials during the ripening processes of fruits. The voluminous work on pectins has been admirably reviewed by Bigelow, Gore, and Howard (*2*) and need not be repeated here. Because of the probable intimate association of changes in the pectin materials with the very important softening of the fruit in storage, however, a brief summary of these changes is desirable.

It is apparent that there is a water-insoluble pectin compound which acts as a cementing material in green fruit, as well as in other fleshy plant tissue. The exact chemical nature of this substance is not established, and it has been mentioned in the literature under various names, as pectose, pecto-cellulose, and calcium pectate. The more recent work indicates that this substance is calcium pectate. This calcium pectate cements the cell walls of the green fruit flesh firmly together, and is largely responsible for the "hardness" of green fruit, since such fruits can be crushed only by actually breaking the cells.

As the fruit ripens, this cementing material slowly hydrolizes, probably by enzymatic action, into substances more readily soluble in water. This material is that generally referred to under the name "pectin," and its formation, which occurs slowly under natural conditions, may be greatly hastened by boiling with water. Pectin, according to some investigators, can be further split by hydrolysis, either with boiling water or by boiling with acids or alkalis. With alkalis, pectic acid is first formed, followed by meta-pectic acid after prolonged boiling. Meta-pectin, a closely similar substance, has also been formed. The products of final hydrolysis have usually been pentose sugars and galactose. More recently Schryver and Haynes (*25*) and Ehrlich (*11*) have worked upon the chemical nature of pectins. The former reported that the pectin obtained from a number of fruits was essentially identical and found the molecule to contain pentose sugars sufficient to account for about one-third its weight. Ehrlich reports pure pectin as consisting primarily of arabinose, galactose, and galacturonic acid.

The changes in the pectins as they occur naturally in the fruits have not been worked out in association with the physical changes in the fruit. The fact seems well established that the cementing material in green fruits is calcium pectate, and that this material accounts for the hardness

of the fruit, since it results in the cells being ruptured when pressure is applied, rather than simply separating. As the cementing material is hydrolized, the cell walls tend to separate more and more readily and the fruit becomes progressively softer. This fact also probably explains the juiciness of green apples, and the apparent lack of juice in the soft or mellow fruit. The juice is primarily within the cells, and escapes only when the cell walls are broken. If the cells can separate readily, the individual cell walls will not be broken to an appreciable extent when the fruit is crushed. Consequently the soft or "mealy" fruit also appears "dry," although actually containing practically as much moisture as when hard. This apparent dryness may also be caused in part by the greater absorption of water by the cell colloids as the fruit softens.

As the fruit ripens, both on the tree and in storage, these changes in pectin materials go on. It has not been established how far they progress, but it is often asserted in the literature that the "pectin decreases as the fruit ripens." This usually has referred to pectin extracted by boiling water, and would include both "pectin" and calcium pectate or "pectose." This would indicate that as the fruit ripens and softens hydrolysis may go so far that some of the pectin materials will break down to sugars. The sugars of the fruit may thus be very slowly augmented throughout the life of the apple. Because of the complexity of the compounds involved and the large quantities of various sugars always present in fruit tissue, these points are very difficult to establish.

SUMMARY OF CHANGES IN THE FRUIT WHILE ON THE TREE

The changes associated with growth and ripening may then be summarized as follows: There is a continuous increase in size of the fruit from blossoming time until late season. This increase will vary greatly with growing conditions. Growth in size is accompanied by changes in the skin, the wax being formed during the latter part of the growing season, and the lenticels being corked over. Associated with these changes are color changes. Red pigment may develop just below the epidermis on fruits exposed to sunlight. The green color gradually gives way to yellowish green or yellow as the chlorophyll disappears. The fruit softens as it ripens, the rate of softening also undoubtedly varying with growing conditions. Associated with all these physical changes, there is a gradual decrease in acidity and an increase in sugars as the fruit ripens. Starch during the late growing season gradually changes over to sugar. Changes in the pectin constituent of the cell walls, consisting probably in insoluble calcium pectate being changed to water-soluble pectin or pectic acid, allows the cells to separate and break more readily and results in a softening of the fruit.

CHANGES IN APPLES FOLLOWING THEIR REMOVAL FROM THE TREE

The removing of fruit from the tree entirely stops certain of the processes going on in the fruit, while others continue in much the same manner as though the fruit remained on the tree. Increase in size stops, as well as development of red color. The latter is probably due to the removal from direct exposure to light rather than to the cutting off of any substance derived from the tree. The green color of the unblushed surface continues to disappear, and the yellow color, previously masked by the green, becomes predominant. It is not known whether or not any changes in the epidermal covering occur following

picking. Softening, already under way while the fruit was on the tree, proceeds rapidly after removal from the tree, unless the fruit is placed at once in a low temperature.

The chemical changes in progress when the fruit is picked from the tree continue mainly in the same direction after picking, though the rate of the various changes may not be the same. From time of picking forward, there is a continuous drop in acidity. Starch present in the fruit quickly changes to sugar, as has been shown by the work of Bigelow, Gore, and Howard (*2*), of Otto (*22*), of Magness and Burroughs.[5] Following completion of this change, there is little further variation in total sugars, although there is undoubtedly a very small loss due to respiration. Even this loss may be partially replaced by acquisitions from the pectin materials. Bigelow, Gore, and Howard found that as the fruit was held in storage there was a continual decrease in the percentage of sucrose and a corresponding increase in reducing sugar. Pectin changes, associated with the softening of the fruit, continue throughout the storage season.

EFFECT OF STORAGE CONDITIONS UPON CHANGES IN FRUIT FOLLOWING PICKING

Among the conditions in storage houses that are within the control of the operator or fruit handler may be included the factors of temperature, humidity, air renewal, or ventilation, air stirring or movement within the room, and the type of package in which the fruit is packed. These factors have been studied in relation to their effect upon certain of the changes occurring in the fruit while in storage. The studies have consisted primarily in determining the effect of these different factors upon the rate of softening of the fruit, upon the acidity changes in the fruit, and upon the general metabolic activity as measured by respiratory activity. Shrinkage in weight, due to loss of moisture from the fruit and loss through respiratory activity, has also been studied.

EFFECT OF TEMPERATURE OF STORAGE UPON RATE OF SOFTENING

Magness and Burroughs[6] measured the rate of softening of eight varieties of apples under different storage conditions. Composite curves, from their data on Jonathan, Delicious, Rome Beauty, Winesap, Baldwin, York Imperial, Esopus (Spitzenberg), and Yellow Newtown, at 36° and 32° are reproduced in Figure 5. It will be noted that the rate of softening in storage was approximately exactly twice as rapid at 36° as at 32° F., fruit at 36° being as soft after two months in storage as fruit at 32° was at the end of four months.

Figures 6 to 11, inclusive, show the relative rates of softening of Delicious, Rome Beauty, Ben Davis, Rhode Island Greening, Winesap, and York Imperial, respectively, when held at 70° F. and at 32° F. Figures 6, 7, and 8, for the first three named varieties include data on fruit picked at three different intervals, approximately two weeks apart.

It is at once apparent that softening in all varieties was greatly accelerated by higher temperatures. However, it is also apparent that there is a distinct difference in the response of different varieties to the various temperatures.

[5] MAGNESS, J. R., and BURROUGHS, A. M. APPLE STORAGE INVESTIGATIONS. The Marble Laboratory, Inc. 2d Rept. Not yet published.
[6] MAGNESS, J. R., and BURROUGHS, A. M. OP. CIT.

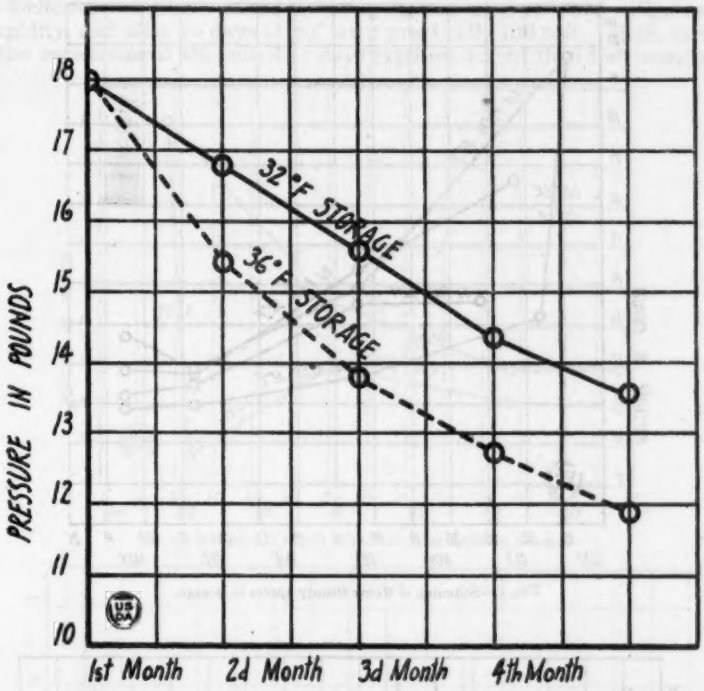


FIG. 5.—Softening rates of apples in storage. Composite curves of Jonathan, Delicious, Rome Beauty, Winesap, Baldwin, York Imperial, Esopus (Spitzenberg), and Yellow Newtown. After Magness and Burroughs.

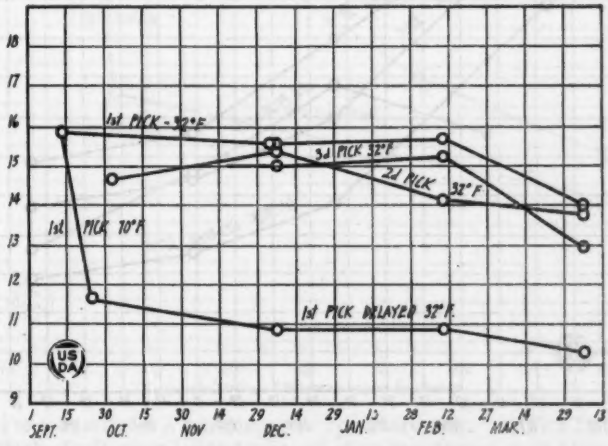


FIG. 6.—Softening of Delicious apples in storage.

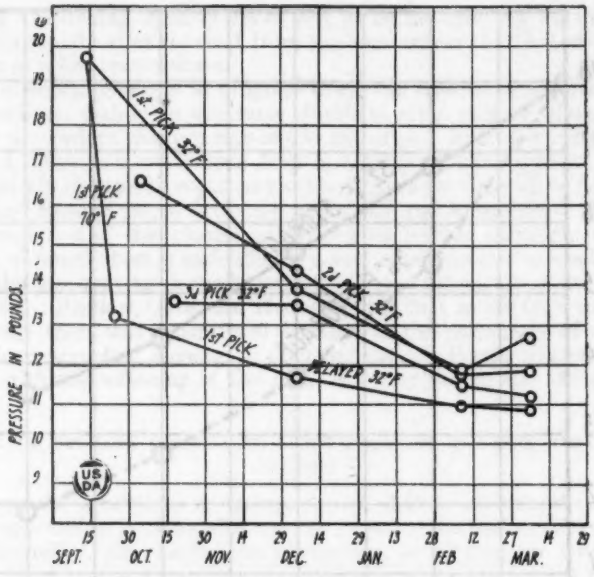


FIG. 7.—Softening of Rome Beauty apples in storage.

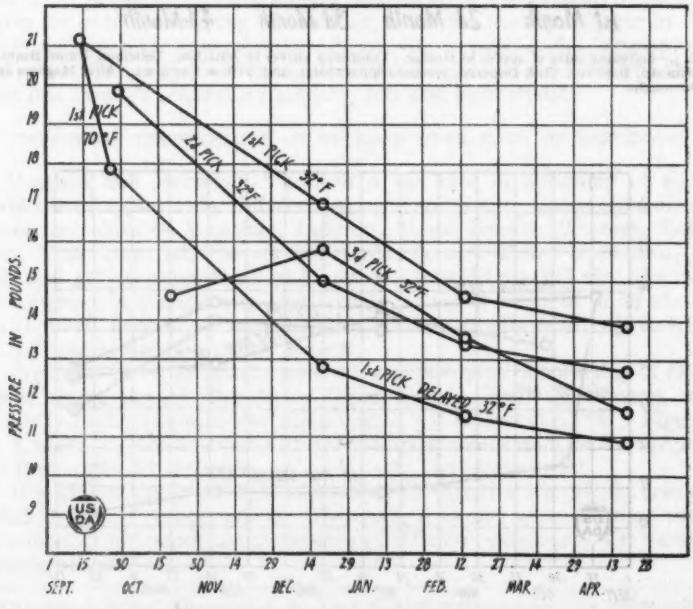


FIG. 8.—Softening of Ben Davis apples in storage.

Delicious and Rhode Island Greening (figs. 6 and 9) softened with great rapidity, and after 10 days at 70° were practically full soft. Both varieties were softer at the end of 12 days exposure to 70° than they were at

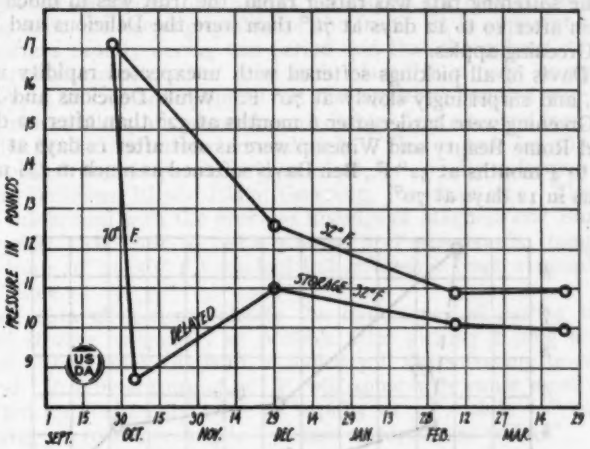


FIG. 9.—Softening of Rhode Island Greening apples in storage.

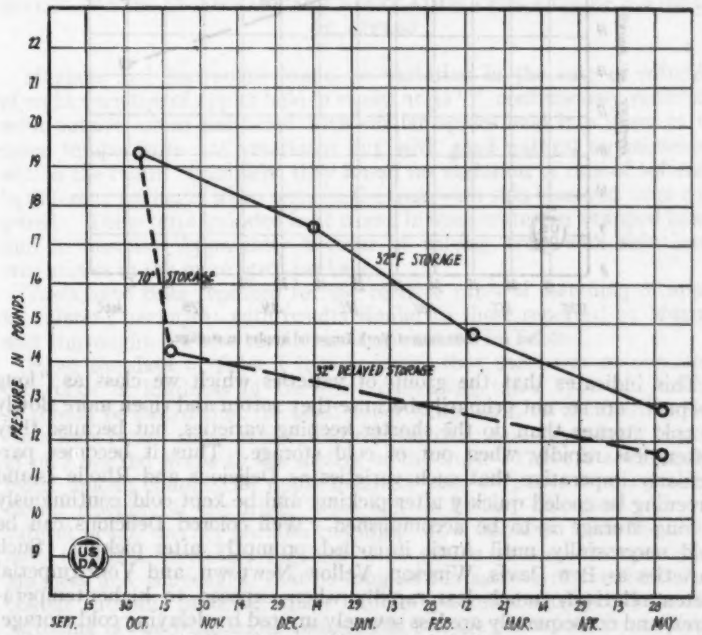


FIG. 10.—Softening of Winesap apples in storage.

the end of 6 months at a continuous temperature of 32°. With Delicious particularly, fruit placed directly in 32° storage softened very little, even after several months in storage. Rhode Island Greening softened more

rapidly in 32° temperature than did Delicious and also softened with great rapidity at 70°.

Rome Beauty and Winesap were somewhat harder when picked, and while the softening rate was rather rapid, the fruit was in much better condition after 10 to 12 days at 70° than were the Delicious and Rhode Island Greening apples.

Ben Davis of all pickings softened with unexpected rapidity in cold storage, and surprisingly slowly at 70° F. While Delicious and Rhode Island Greening were harder after 6 months at 32° than after 12 days at 70°, and Rome Beauty and Winesap were as soft after 12 days at 70° as after 3 to 4 months at 32° F., Ben Davis softened as much in 2½ months at 32° as in 12 days at 70°.

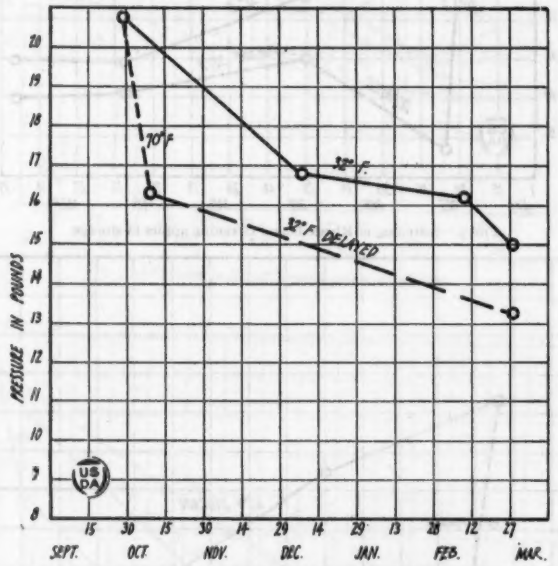


FIG. 11.—Softening of York Imperial apples in storage.

This indicates that the group of varieties which we class as "long keepers" are so, not primarily because they soften and ripen more slowly in cold storage than do the shorter keeping varieties, but because they soften less rapidly when out of cold storage. Thus it becomes particularly imperative that such varieties as Delicious and Rhode Island Greening be cooled quickly after picking, and be kept cold continuously if long storage is to be accomplished. Well colored Delicious can be held successfully until April if cooled promptly after picking. Such varieties as Ben Davis, Winesap, Yellow Newtown, and York Imperial soften relatively much less rapidly when exposed to higher temperatures, and consequently are less severely injured by delaying cold storage. All varieties, however, soften very much more rapidly at any temperature above 32° than at that temperature.

EFFECT OF DELAYED STORAGE UPON HARDNESS OF THE FRUIT

A study of the data presented in Figures 6 to 11, inclusive, is interesting from the standpoint of the effect of delaying the time of storing after picking upon the keeping quality of the fruit. It is apparent that the fruit softens rapidly during this period and that throughout its storage life it remains much softer than fruit placed in storage immediately. Ramsey, et al. (*24*) have pointed out that apples so handled were usually softer, more yellow, and showed more decay when removed from storage than did similar fruit stored immediately. This is true for all varieties here studied, but is particularly marked for rapidly softening apples, such as Delicious, Rhode Island Greening, Jonathan, etc. These data are in full accord with the previous findings of Magness and Burroughs, who report that fruit in cellar storage in Pennsylvania (temperature range from 40° to 50° F.) reached full softness in from a month to six weeks' time.

These data all indicate clearly the importance of getting the fruit to cold storage as quickly as possible after picking if long holding is desired. Only when the fruit is cooled will the softening processes be checked. A temperature of 36° F. will apparently cause most varieties to soften approximately twice as rapidly as 32°, while 70° will cause softening to proceed from 5 to 15 times as rapidly as will 32°.

EFFECT OF TYPE OF PACKAGE AND VENTILATION UPON RATE OF SOFTENING OF APPLES

Magness and Burroughs found no variation in the rate of softening of eight varieties of apples held in rooms at 32° F. continuously ventilated with outside air as compared with similar apples held in a room at the same temperature not ventilated but with good natural air movement within the room. Similarly, they found no variation in rate of softening in different packages when tests on the fruit with skin removed were compared. These tests included fruit stored in open crates, in wrapped boxes, and in barrels. Apparently because of wilting, fruit with skin intact was harder in the more open packages.

Tests have been repeated for the relative rates of softening of apples in different packages; with results similar to those reported by Magness and Burroughs. These results are summarized in Table I.

From the data in Table I it is apparent that there was no consistent difference in hardness of the fruit depending upon type of package. It should be stated that the oiled paper wraps used on the fruit in these tests were rather small in size, and that there was no appreciable transfer of oil from the wraps to the surface of the fruit. There is a widespread belief among commercial apple handlers that oil paper wraps result in slower ripening of the fruit. This will be further discussed under the subject of oil, wax, or paraffin coatings. It is apparent from the results here recorded, however, together with results previously reported by Magness and Burroughs, that so long as the surface of the fruit is not coated with any material which inhibits gaseous exchanges the rate of softening of the fruit under commercial storage conditions is not appreciably affected by ventilation, type of package, etc. Temperature apparently is the one controlling factor in determining the rate of softening of the fruit, if held under conditions of normal respiration.

TABLE I.—*Effect of type of package upon the rate of softening of apples in 32° F. storage*

Variety.	Package.	Average pressure test when stored.	Average pressure test at end of storage season.
		Pounds.	*Pounds.*
Delicious	Barrel	15. 20	13. 47
Do	Oil paper wrapped box	15. 20	14. 47
Do	Non-oil paper wrapped box	15. 20	13. 72
Rome Beauty	Barrel	18. 16	12. 23
Do	Oil paper wrapped box	18. 16	11. 70
Do	Non-oil paper wrapped box	18. 16	12. 42
Ben Davis	Barrel	20. 52	13. 63
Do	Oil paper wrapped box	20. 52	12. 95
Do	Non-oil paper wrapped box	20. 52	12. 27
York Imperial	Barrel	20. 75	14. 70
Do	Non-oil paper wrapped box	20. 75	14. 85
Rhode Island Greening	Barrel	17. 13	10. 99
Do	Oil paper wrapped box	17. 13	11. 07
Do	Non-oil paper wrapped box	17. 13	10. 79
Winesap	Barrel	19. 33	12. 88
Do	Non-oil paper wrapped box	19. 33	12. 74

EFFECT OF TEMPERATURE AND TYPE OF STORAGE PACKAGE UPON ACIDITY

Acidity is of marked importance in determining the eating and cooking quality of fruits. For this reason changes in acidity while the fruit is in storage are of much importance in determining quality in stored fruit.

Gerber (*12*) reported that the acidity disappeared from apples with great rapidity when the apples were held at 30° C. (86° F.) and believed that it was consumed by the respiratory processes of the fruit. At 18° C. (64 2/5° F.) acidity disappeared more slowly, and at lower temperatures (0° C. or 32° F.) it remained practically constant. Other workers, using other fruits, however, have obtained somewhat different results. Hawkins and Magness (*16*) and Hawkins (*15*) on grapefruit, Diehl and Magness (*10*) on plums, and Magness (*19*) on Bartlett pears, all found that the acidity decreased when the fruits were held at low temperatures, but remained practically constant, or in the case of Bartlett pears and plums actually increased, when the fruit was stored at temperatures of about 60° F. Bigelow, Gore, and Howard (*2*) found a constant decrease in acidity when apples were removed from 32° storage and held at 60° F. or 70° F. Magness and Burroughs [7] found acidity to decrease most rapidly in apples stored in a cellar (40° to 50° F.), less rapidly when stored at 35° F., and still less rapidly when held at 32° F.

Figures 12 and 13 show changes in acidity in the six varieties of fruit here studied both during the time they were in storage at 32° F. and during an initial period of 12 days before certain lots were placed in storage.

Acidities were determined by cutting plugs of apple tissue with a cork borer, preparing these in a finely divided condition by passing through a sampling press of the type described by Clark (*6*), weighing out 100 grams, boiling and making up to 1,000 cubic centimeters volume with distilled water. Samples were preserved with toluol, and filtered and titrated after three days' extraction. For titration, N/10 NaOH

[7] MAGNESS, J. R., and BURROUGHS, A. M. OP. CIT.

was used with phenolphthalein as indicator. The results are expressed in cubic centimeters of N/10 acid per 10 grams of wet tissue.

There is a constant, but slow, decrease in acidity in all varieties during the storage season. Total decrease is very nearly the same for all varieties, regardless of total acidity present. Thus Delicious, with very low acidity at the start of the season, lost acid about as rapidly as Rhode

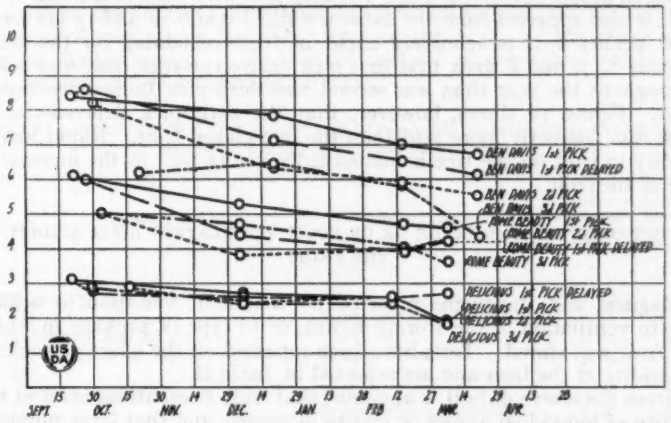

BEN DAVIS 1st PICK
BEN DAVIS 1st PICK DELAYED
BEN DAVIS 2d PICK
BEN DAVIS 3d PICK
ROME BEAUTY 1st PICK
ROME BEAUTY 2d PICK
ROME BEAUTY 1st PICK DELAYED
ROME BEAUTY 3d PICK
DELICIOUS 1st PICK DELAYED
DELICIOUS 1st PICK
DELICIOUS 2d PICK
DELICIOUS 3d PICK
USDA
SEPT. OCT. NOV. DEC. JAN. FEB. MAR. APR.

FIG. 12.—Acidity changes in Ben Davis, Rome Beauty, and Delicious apples while in storage. Acid as cc. N/10 in 10 gm. wet tissue.

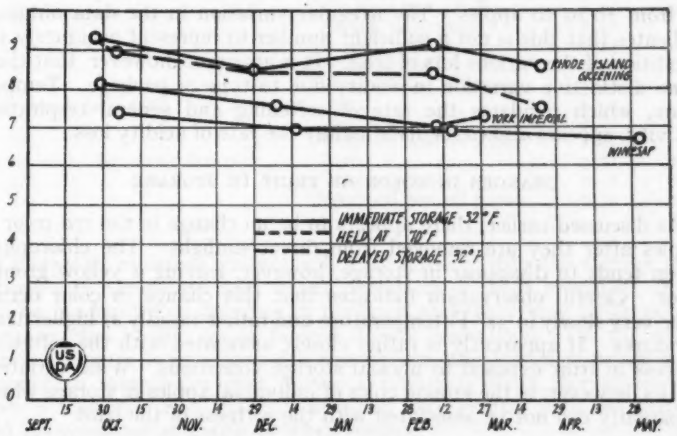


FIG. 13.—Acidity changes in Rhode Island Greening, York Imperial, and Winesap apples while in storage. Acid as cc. N/10 in 10 gm. wet tissue.

Island Greening, which was very high in acid content. Total loss in Delicious amounted to more than one-third the amount originally present, while loss in Rhode Island Greening was only about one-ninth the total.

Loss in acidity during an initial period in warm storage was relatively slight in all varieties, and in some varieties there was an apparent gain, due probably to variation in the samples. It is apparent, however, that

acidity decreased relatively less rapidly during exposure to warm temperatures than did softening of the fruit. It would seem from these data that fruit ripened rapidly at high temperatures would be higher in acidity for the same degree of softness than would the same fruit ripened more slowly at lower temperatures. Thus it appears that while apples will lose acidity when held at temperatures of around 70° F., this loss is less rapid in proportion to the other ripening processes than it is at 32° F.

It is also apparent from the data on which Figures 12 and 13 are based that acidity loss is relatively rapid in fruit remaining on the trees. Figures 6, 7, and 8 show that first-pick delayed-storage fruit was softer throughout the year than was second and third pick immediate-storage fruit. Figure 12 shows, however, that the early-pick delayed-storage fruit was distinctly more acid than the late-picked fruit. Rapid loss in acidity in fruit on the tree is undoubtedly due in part to the increase in size of the fruit.

EFFECT OF TYPE OF PACKAGE OR OF ROOM VENTILATION UPON ACIDITY OF THE FRUIT

Magness and Burroughs reported no consistent variation in acidity due to ventilation of the storage rooms, or to type of package in which the fruit was stored. Tests have been repeated on the effect of package on acidity of the fruit and are reported in Table II.

From the above data it is apparent that wide fluctuations exist in the acidity of individual apples or groups of apples, and that large numbers must be used if a figure representing the correct average is to be obtained. Individual figures in the above tables are based upon analysis of from 10 to 20 apples. The irregular variation in the data obtained indicates that this is not a sufficient number to represent accurately the condition of the various lots of fruit. It is apparent, however, that there is no distinctive variation in acidity due to type of package. Temperature, which regulates the rate of softening and general respiratory activity, appears also to regulate mainly the rate of acidity loss.

CHANGES IN COLOR OF FRUIT IN STORAGE

As discussed earlier, there appears to be no change in the red color of apples after they are removed from direct sunlight. The chlorophyll green tends to disappear in storage, however, leaving a yellow ground color. Careful observation indicates that this change in color occurs only very slowly in 32° F. temperature and rather rapidly at higher temperatures. It apparently is rather closely associated with the softening process in fruit exposed to normal storage conditions. Wide variation occurs, however, in the ground color of individual apples in storage which apparently can not be associated with the softness of the fruit.

CHANGES IN SUGARS, PECTINS, MOISTURE CONTENT, ETC., DURING STORAGE

Analysis to determine the chemical changes in the fruit here studied while in storage, other than change in acidity, have not been made. The work of Bigelow, Gore, and Howard (*2*), of Magness and Burroughs,[8] and of numerous other workers has shown that there is a sharp increase

[8] MAGNESS, J. R., and BURROUGHS, A. M. OP. CIT

in sugars after picking, due to starch being transformed to sugar. Following this, there is probably a very slow dropping off in total sugar, due to respiratory activity. This decrease, however, is hardly sufficient to be detected by chemical analysis, because of the wide variation in individual fruits.

Pectin changes in apples while in storage have never been satisfactorily determined, because of the multiplicity of closely related compounds present and the difficulty of determining these compounds quantitatively. It is undoubtedly true, however, that as the fruit softens there is a gradual disappearance of the calcium pectate, or substance which acts as a cementing factor between cell walls.

TABLE II.—*Effect of type of package upon acidity in apples in cold storage—cubic centimeters N/10 acid per 10 grams of wet tissue*

Variety.	Type package.	Acidity beginning of storage season.	Acidity end of storage season.
Rome Beauty, first pick	Barrel	6.10	4.50
Do	Wrapped box	6.10	4.64
Rome Beauty, second pick	Barrel	5.05	4.85
Do	Wrapped box	5.05	4.28
Do	Wrapped box, oil paper	5.05	3.86
Ben Davis, first pick	Barrel	8.38	6.10
Do	Wrapped box, oil paper	8.38	7.28
Do	Basket, unwrapped	8.38	7.73
Ben Davis, second pick	Barrel	8.18	5.88
Do	Wrapped box	8.18	4.95
Do	Wrapped box, oil paper	8.18	5.61
Do	Basket, unwrapped	8.18	5.28
Delicious, first pick	Barrel	3.13	2.27
Do	Wrapped box	3.13	1.82
Do	Wrapped box, oil paper	3.13	1.68
Delicious, second pick	Barrel	2.90	2.01
Do	Wrapped box	2.90	1.64
Do	Wrapped box, oil paper	2.90	2.10
Rhode Island Greening, first pick	Barrel	9.31	7.75
Do	Wrapped box	9.31	8.55
Do	Wrapped box, oil paper	9.31	8.33

Changes in moisture content will vary directly with the storage conditions. If apples are held under conditions of high humidity, there will be practically no decrease in moisture, and there may be an actual increase, due to the water formed during respiration. On the other hand, if storage is in a very dry atmosphere, the loss of moisture may amount to several per cent during a relatively short storage season. Loss of moisture from the fruit also will vary greatly with the variety and the condition of maturity when picked.

LOSS OF MOISTURE AS RELATED TO PACKAGE IN WHICH APPLES ARE STORED

At the time the fruit was placed in storage certain average lots in each package were accurately weighed. These same lots were weighed again at the end of the storage season and the total shrinkage in weight was determined. Humidity in the room ranged from 85 to 90 per cent

of saturation with slow natural air movement. Humidity was sufficiently high so that traces of mold growth were present on the ends of boxes and inside the barrels. Under these conditions there was no shriveling discernible in any fruit, even after a long storage season in open boxes. Losses in weight are summarized in Table III.

TABLE III.—*Effect of type of storage package upon fruit shrinkage*

Variety.	Length of time in storage.	Type of package.	Shrinkage; loss in weight.
	Months.		*Per cent.*
Winesap	7	Barrels, closed	0.6
Do	7	Barrel, open	2.0
Do	7	Wrapped box	1.7
Do	7	Open box, fruits paraffin coated	1.1
Rome Beauty	5	Barrel, closed	.55
Do	5	Barrel, open	.8
Do	5	Wrapped box	.7
Do	5	Wrapped box, oil paper	.7
Do	5	Unwrapped box, fruits paraffin coated	.3
Ben Davis	6½	Barrel, closed	.95
Do	6½	Barrel, open	1.15
Do	6½	Wrapped box	1.5
Do	6½	Wrapped box, oil paper	1.4
Do	6½	Bushel basket, unwrapped	2.0
Do	6½	Unwrapped box, fruits paraffin coated	1.0
Delicious	6½	Barrel, closed	.65
Do	6½	Barrel, open	1.25
Do	6½	Wrapped box	1.00
Do	6½	Wrapped box, oiled paper	1.00
Do	6½	Bushel basket, unwrapped	2.00
Do	6½	Unwrapped box, fruits paraffin coated	.65
Rhode Island Greening	6	Barrel, closed	1.0
Do	6	Wrapped box	2.3
Do	6	Wrapped box, oiled paper	2.0

The data recorded in Table III are of primary interest in showing that if the storage room is kept sufficiently moist very little loss in weight will occur regardless of the type of package used. The storage season varied from five months for Rome Beauty to seven months for Winesap, yet the maximum loss in weight in open packages was only slightly over 2 per cent. This includes both moisture from the fruit and gaseous-respiratory products. Based upon the results of respiration tests, the loss in weight of apples due to respiration during six months at 32° F. would be from 0.2 to 0.3 per cent of the total weight. Hence all loss above 0.3 per cent may be attributed to moisture loss from the fruit. Part of this moisture may, however, be that formed by the respiration of the fruit.

Maximum losses in all varieties occurred in the most open containers. Closed barrels showed losses of less than 1 per cent, wrapped boxes somewhat more, and unwrapped baskets still higher shrinkage. Fruit coated thinly with paraffin showed very slight shrinkage, even in open containers.

While the losses in weight here reported were all slight, that will be true only when the room as a whole is kept humid. If the air becomes dry, heavy shrinkage will result, particularly in fruit held in open containers.

If fruit is stored in closed barrels, the humidity of the storage room is not a very important factor. In open barrels or boxes, however, it is important to keep the humidity up to at least 85 per cent, to avoid shriveling the fruit. In more open containers, as baskets or slatted crates, it is very essential that humidity be kept sufficiently high.

RELATION OF TIME OF PICKING TO LOSS IN WEIGHT

Tests of the rate of loss of weight in storage as related to time of picking were made upon the three varieties, Ben Davis, Rome Beauty, and Delicious. There was no distinctive variation in loss of weight that could be associated with time of picking with the picking dates here used, namely, September 14 to October 10. It is a well known fact that fruit picked when extremely immature will wilt more readily than will well ripened fruit, but apparently the skin of the apples here studied was sufficiently cutinized by September 14 to prevent excessive moisture loss.

EFFECT OF COATING THE SURFACE OF THE FRUIT WITH OIL OR PARAFFIN UPON THE RIPENING PROCESSES OF THE FRUIT

During the normal respiration of fruit, oxygen is taken up from the air, and carbon dioxid given off from the fruit. The carbon dioxid is generated throughout the tissue, penetrates to the epidermis, probably largely through the intercellular spaces, which are relatively large in apple tissue, and passes out through the epidermis, while oxygen enters by a similar route. Consequently, the condition of the epidermis is of great importance in determining the concentrations of CO_2 and of O_2 in the tissues within the fruit.

In order to determine the effect of coating the surface of the fruit, thereby reducing the permeability of the epidermis, certain lots of all varieties were coated when put in storage. Certain fruit was coated lightly with a non-drying, non-odorous oil, by wiping the surface of the fruit with a well oiled cloth. Other lots were coated with paraffin by wiping them with a solution of paraffin in a volatile solvent, the preparation being one that has been widely advertised for applying to fruit in order to decrease wilting, to improve keeping quality, and to improve general market appearance. Fruits treated both with paraffin and with oil coating, and also check fruits, untreated, have been tested at intervals for hardness, for acidity, for color and general appearance, and for flavor. Respiration experiments in which both oxygen absorbed and carbon dioxid given off were measured, have been carried out at various temperatures.

Results of tests on the rate of softening of apples after coating with paraffin or with oil are somewhat variable, depending upon the variety used. At the end of the storage season coated Delicious apples were about as the checks which received no coating in hardness. Rome Beauty coated fruit was slightly harder than the check apples, York Imperial and Ben Davis were distinctly harder, and Winesap showed even a greater difference in hardness at the end of the season. This difference in hardness of the fruit was apparent when the fruit was withdrawn from 32° F. storage, and became more marked after the fruit had stood for 10 days at a temperature of 60° to 70° F. Apples used as checks were similar in all ways to coated lots, except that they were not coated with any compound. Retardation of softening apparently varied directly with the quantity of oil paraffin on the fruit. If a very heavy coating was applied, the retardation was distinctly greater than if only a light coating was present.

EFFECT OF COATING UPON THE COLOR CHANGES OF THE FRUIT

Much more marked than the influence of coating the surface on the rate of softening of apples was its influence upon color. Coated fruit showed very little change in the intensity of its green color during the time it was in storage. At the end of the storage season such fruit was very nearly the same shade of green as at the time of placing in storage.

EFFECT OF COATING UPON THE ACIDITY OF THE FRUIT

Determinations for acidity were made upon coated fruit and upon control fruit throughout the storage season, and there was no consistent variation in acidity due to coating the fruit. Certain varieties showed somewhat higher acidity in coated fruit at the end of the season and others showed less acidity. Because of the wide variation in acidity in individual apples it would be necessary to use large samples to determine accurately whether coating does influence the rate of acidity loss, and fruit was not available in sufficient quantities to determine this more accurately.

EFFECT OF COATING UPON THE FLAVOR OF THE FRUIT

The flavor of apples that had been coated either with oil or with paraffin varied greatly with the quantity of the coating material present. Unless very heavy coatings had been used, the fruit was of good quality when removed from storage at 32° F. If heavy coating had been used, however, the fruit was stale, flat, and of distinctly unpleasant flavor when removed from 32° F. storage.

After holding for 10 days at a temperature of 60° to 70° F., much of the fruit that was of good quality when removed from a 32° F. room developed undesirable flavors, becoming stale and fermented in taste. Certain varieties, particularly Rome Beauty, became entirely inedible. Some of the very lightly coated fruits maintained a good flavor throughout the period of the tests. These were the fruits that showed a minimum retardation of softening and of color change, and obviously carried very little of the coating material.

The tests so far carried out demonstrate clearly that the ripening processes of apples may be retarded by coating the fruit with substances that reduce the permeability of the epidermis. This retardation is at the expense of flavor, however, unless the coatings be very light. Coatings so light as not to injure the flavor of the fruit will cause only a very slight retardation of the softening process, with a somewhat greater effect upon the color of the fruit.

RESPIRATION OF COATED AND NORMAL APPLES AT DIFFERENT TEMPERATURES

In order to determine the causes of the retardation of ripening in coated fruit, as well as the cause of the development of bad flavor that may accompany this retardation, the respiration of certain lots of fruit was measured. It is important that both oxygen absorption and CO_2 output be determined, to arrive at a real measure of what is going on in the fruit. In the absence of oxygen, fruit will still give off CO_2 by anerobic respiration, as has been shown by Hill (17) and others. Consequently, the measurement of the oxygen absorption is essential to a knowledge of the source of the CO_2 given off by the fruit.

METHOD OF ESTIMATING CO_2 OUTPUT AND O_2 INTAKE

Most of the work in which both O_2 absorption and CO_2 output have been studied has been carried out by placing fruit in closed chambers, and analyzing the included atmosphere for O_2 and CO_2 at the end of a certain interval. This results in the fruit being exposed to an atmosphere higher in CO_2 pressure and lower in O_2 pressure than the air. The work of Kidd and West (*14*), as well as investigations reported here, has demonstrated the importance of CO_2 and O_2 concentrations in the ripening of apples.

In order to determine both CO_2 given off and O_2 absorbed without exposing the fruit to varying concentrations of these two gases, the apparatus shown in Figure 14 was used. A large bottle containing a water reserve (A) is connected by a double siphon feed of glass tubing to a second bottle (B). The siphon feed maintains a constant water

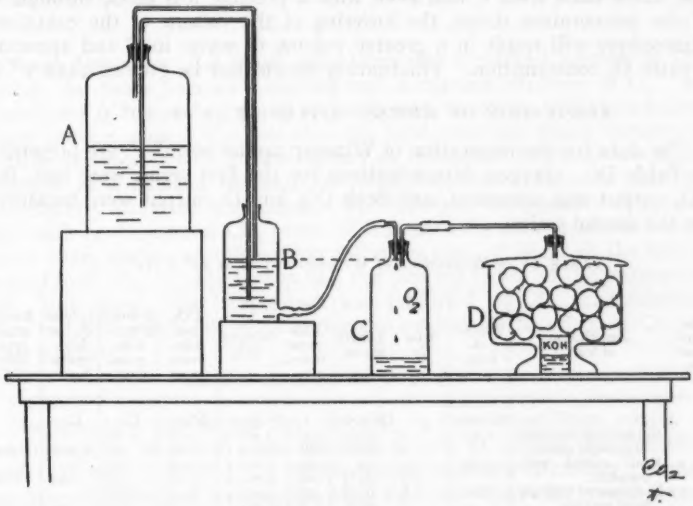


FIG. 14.—Apparatus for determining the amount of CO_2 given off and the amount of O_2 absorbed by the fruit.

level in bottle B. Bottle B has a tubulature at the bottom, by which it is connected, through rubber and glass tubing, to a third bottle, C. Bottle C is full of pure O_2, being filled by displacement of water. A second tube from bottle C is connected into the closed desiccator D which contains the fruit. In the bottom of the desiccator D is placed a wide beaker containing KOH, a solution approximately twice normal being used.

As the fruit respires O_2 is absorbed by it and CO_2 is given off. The CO_2 given off in the desiccator D is absorbed in the KOH solution in the bottom of the desiccator; a negative pressure consequently develops in the desiccator, due to loss of O_2. As a result pure O_2 passes over from C, and the loss in O_2 in bottle C is replaced by water which flows from bottle B. It is important that the height of the water level in B be so adjusted that it will be just level with the end of the connection

into bottle C. If this is done, the whole equipment will be under atmospheric pressure at all times.

The oxygen consumption was measured by measuring the volume of water in bottle C and subtracting 3 per cent from this volume as an estimation of the quantity of oxygen absorbed in the water. CO_2 dissolved in the KOH solution was determined by the double titration method as used by Gore (*13*), phenolphthalein and methyl orange being the indicators used.

Analyses of the contained air in desiccator D showed an accumulation of CO_2 of less than 1 per cent by volume even when held at high temperatures and with rapid respiration going on. The apparatus can be used at any temperature by substituting NaCl or $CaCl_2$ solutions for the water at temperatures below 32° F.

It is essential that the temperature be kept constant during any run. If the temperature rises, the atmosphere in D and C will expand, driving the water back from C and even with a possible loss of O_2 through B. If the temperature drops, the lowering of the volume of the contained atmosphere will result in a greater volume of water in C and apparent greater O_2 consumption. Fluctuation should not be greater than 1° C.

RESPIRATION OF WINESAP APPLES AT 32° F. (0° C.)

The data for the respiration of Winesap apples at 32° F. are presented in Table IV. Oxygen determinations for the first series were lost, but CO_2 output was measured, and both CO_2 and O_2 output were measured for the second series.

TABLE IV.—*Respiration of Winesap apples at 32° F. (0° C.)*

Experiment No.	Description of fruit.	Weight of fruit.	Run No.	Length of run.	Kilogram hours.	Weight CO_2.	CO_2 per kilogram hour.	Volume CO_2 per kilogram hour.	Volume O_2 per kilogram hour.	Respiratory ratio $\frac{CO_2}{O_2}$.
		Grams.		*Hours.*		*Mgm.*	*Mgm.*	*Cc.*	*Cc.*	
1	Normal fruit 5 months in storage; check.	2,873	1	358½	1,030.0	2,167.5	2.10	1.07	.....	.....
			2	381½	1,096.0	2,007.1	1.83	.93	1.02	0.91
2	Fruit coated with paraffin.	2,907	1	358	1,040.7	1,807.7	1.74	.89	.....	.....
			2	381½	1,109.0	1,625.9	1.47	.75	.93	.81
3	Fruit coated with oil, light coating.	2,822	1	357½	1,008.9	1,745.4	1.73	.88	.....	.....
			2	381½	1,076.6	1,623.3	1.51	.77	.97	.79
4	Fruit coated with oil, very heavy coating.	2,801	1	357	1,000.0	1,183.6	1.18	.60	.....	.....
			2	381½	1,068.6	1,188.6	1.11	.56	.....	.....

All of the fruit used in experiments 1 to 4 was closely comparable except for the treatments received. It is at once apparent that respiration was markedly reduced by the coatings which the fruit in experiments 2, 3, and 4 received. Light oil coating and paraffin coating both resulted in a marked decrease in CO_2 output as compared with the control, or untreated, fruit. Furthermore, the fact is developed that in the case of these fruits the CO_2 output was not limited by the oxygen supply, for in the coated fruit there was a greater consumption of oxygen per unit of CO_2 output than in the case of the control fruit. This is shown by comparing the respiration ratio, obtained by dividing the volume of CO_2 per unit time and weight of fruit by the volume of O_2 absorbed. The heavily oiled fruit showed an even greater decrease in CO_2 output than did the lightly oiled fruit. Unfortunately, the oxygen absorption record was lost for these heavily coated apples.

At the end of the run samples of the atmosphere from the interior of the apples (from intercellular spaces) were extracted and analyzed. The method employed was that previously described by one of the writers (*20*). These data, which show the actual atmospheric conditions prevailing inside the fruit, where the respiratory activity takes place, are recorded in Table V.

TABLE V.—*Analyses of atmosphere from intercellular spaces of normal and coated Winesap apples at 32° F. (0° C.)*

Experiment No.	Per cent CO_2.	Per cent O_2.	Per cent N_2 by difference.
1. Control; no treatment	2.3	18.4	79.3
2. Medium paraffin coating	11.1	12.6	76.3
3. Light oil coating	10.1	10.4	79.5
4. Heavy oil coating	12.4	2.9	84.7

These data show that in all cases there was a marked increase in CO_2 within the tissue following coating and a marked decrease in O_2. An abundance of oxygen appeared to be present, however, in all lots except those heavily oiled. The fact that the light coatings, which resulted in a large increase of CO_2 in the tissues as compared to the controls, also resulted in a marked decrease in respiratory rate indicates that the CO_2 directly inhibited respiration. The fact that the quantity of O_2 used per cubic centimeter of CO_2 given off was actually greater in the coated fruits than in the controls further indicates that, at least in the lightly coated fruit, the O_2 supply was not the limiting factor in the respiration rate, but rather that respiration was inhibited by CO_2 accumulation. This will be further discussed under the subject of the effect of CO_2 on fruit ripening.

RESPIRATION AT 64.5° F. (18° C.)

Magness (*20*) has shown that the CO_2 in the intercellular spaces of normal apples increases when the fruit is held at higher temperatures, and that the O_2 within the tissues correspondingly decreases. Consequently, the effect of coating the fruit with oil or paraffin would appear to be accentuated at higher temperatures. CO_2 output and O_2 intake were measured on three varieties, and after various treatments, while being held at 64.5° F. (18° C.). Results of these respiration tests are recorded in Table VI.

The data reported in Table VI are of much interest in interpreting the exact effect of coating the surface of the fruit with substances reducing permeability to gases. It will be noted that all cases of coating the fruit (experiments 2, 6, 11, and 12) resulted in greatly decreased CO_2 output, and greatly decreased O_2 absorption as well. Metabolic activity in these lots was reduced to only slightly over half that shown in check lots of fruit. This is in accordance with the slower softening and longer retention of green color in these coated fruits. Also of very marked interest is the respiratory ratio, or ratio of CO_2 given off to O_2 taken up by various lots of fruit. Gerber (*12*) reported that at 18° C. the respiratory ratio was practically unity in apples. In other words, one molecule of CO_2 was given off per molecule of O_2 absorbed. This would be the case if reduc-

ing sugars were being completely oxidized to CO_2 and H_2O. On the other hand, if O_2 is not available, CO_2 will still be formed, as a result of anerobic respiration, or alcoholic fermentation, with a resulting formation of certain products that give a bad flavor to the fruit. The normal oxidation of organic acids to CO_2 and H_2O will also result in a respiratory ratio superior to 1.

TABLE VI.—*Respiration of apples at 64.5° F. (18° C.)*

Experiment No.	Description of fruit.	Weight of fruit.	Run No.	Length of run.	Kilogram hours.	CO_2 given off.	CO_2 per kilogram hour.	Volume CO_2 per kilogram hour.	Volume O_2 per kilogram hour.	Respiration ratio $\frac{CO_2}{O_2}$
		Gm.		*Hours.*		*Mgm.*	*Mgm.*	*Cc.*	*Cc.*	.....
1	Rome Beauty; early pick, (Sept. 14,) held at 32° in unwrapped box until Dec. 18.	2,338	1	71⅝	167.6	2,947.7	17.6	8.96	7.88	1.14
			2	93¾	219.3	4,292.3	19.6	9.98	9.51	1.05
			3	94¾	221.5	4,077.0	18.4	9.37	8.28	1.13
2	Rome Beauty; fruit similar to experiment 1; lightly coated with mineral oil.	2,240	1	71	159.0	1,711.5	10.8	5.50	.....	.....
			2	93⅛	209.6	1,994.2	9.5	4.84	.....	.....
			3	95¼	213.4	1,844.7	8.6	4.38	4.33	1.01
3	Rome Beauty; similar to experiments 1 and 2 except held in oiled paper wraps.	2,218	1	70½	156.4	2,646.3	16.9	8.60	7.70	1.12
			2	93¼	207.9	3,828.0	18.4	9.37	9.38	1.00
			3	95¾	212.4	3,728.3	17.5	8.91	8.85	1.01
4	Rome Beauty; late pick (Oct. 17); well colored and rather soft	2,471	1	69⅛	170.9	2,253.6	13.2	6.71	5.27	1.28
			2	94½	233.5	3,443.3	14.7	7.48	7.41	1.01
			3	96½	238.5	3,492.3	14.6	7.43	7.40	1.00
5	York Imperial; from oil paper wrapped box held at 32° F. 3 months.	2,569	1	76	195.3	2,553.5	13.0	6.62	.....	.....
			2	94⅛	243.8	2,954.0	12.1	6.16	5.17	1.19
			3	116½	298.9	3,359.8	11.2	5.70	5.64	1.01
6	York Imperial; similar to experiment 5, but oil coated when put in 32° storage.	2,528	1	96¼	244.6	2,180.4	8.9	4.53	3.58	1.21
		2,404	2	95⅛	228.8	1,841.6	8.05	4.10	3.08	1.33
			3	116½	280.1	1,949.1	6.9	3.51	3.62	.99
7	York Imperial; similar to experiments 5 and 6, held in ordinary wrapped box.	2,580	1	96½	249.0	3,363.7	13.5	6.87	6.93	.99
			2	95½	246.0	2,893.2	11.8	6.01	5.51	1.09
			3	117¾	301.2	3,327.1	11.0	5.60	5.78	.97
8	York Imperial; barrel storage.	2,609	1	96½	251.8	3,313.7	13.2	6.72	6.61	1.01
			2	95¼	248.9	2,695.8	10.9	5.55	5.34	1.04
			3	116¾	304.6	3,289.0	10.8	5.50	5.37	1.02
9	York Imperial; unwrapped box storage.	2,667	1	96⅛	256.5	3,274.9	12.8	6.52	6.53	1.00
			2	95¼	255.8	2,942.1	11.1	5.65	5.39	1.05
			3	116¼	294.7	3,352.8	11.4	5.80	5.85	.99
10	Winesap; held 3 months at 32° F.; unwrapped box.	2,709	1	98	265.5	4,240.9	16.0	8.14	8.20	.99
			2	116½	315.3	5,372.8	17.0	8.65	8.04	1.07
11	Winesap; similar to experiment 10, but oil-coated when removed to 64.5° F.	2,606	1	97½	254.1	2,756.3	10.8	5.50	5.16	1.07
			2	117	305.1	3,308.4	10.8	5.50	4.51	1.22
12	Winesap; fruit paraffin-coated when put in at 32°.	2,298	1	98⅛	226.4	2,172.6	9.6	4.89	4.48	1.09
			2	116⅛	266.1	2,776.4	10.4	5.29	4.27	1.24
13	Winesap; held in wrapped box; no treatment.	2,723	1	96¼	262.1	4,184.7	16.0	8.14	8.07	1.01
			2	118½	323.4	5,439.0	16.8	8.55	7.82	1.09

TABLE VII.—*Composition of intercellular atmosphere of Rome Beauty apples held at 64.5° F. (18° C.)*

Respiration experiments No.	Description of fruit.	CO_2.	CO_2.	Per cent N_2 by difference.
		Per cent.	*Per cent.*	
1	Early pick, untreated	4.95	14.7	80.35
2	Early pick, oil coated	15.8	1.9	82.3
4	Late pick, colored ripe	6.8	15.5	77.7

At the temperature here studied (64.5° F. or 18° C.) the respiratory ratio in all untreated fruit was fairly close to unity, averaging slightly above. Coated fruit averaged a somewhat higher respiratory ratio. Experiments 6, 11, and 12 show an average ratio of 1.16, while checks for York Imperial and Winesap runs averaged 1.04. Data on the O_2 absorption of oil-coated Rome Beauty apples were lost. Consequently, at 64.5° F. it appears that the oxygen supply was somewhat depleted in coated fruits as compared to untreated fruit, and that there was the beginning of anerobic respiration with resulting bad flavor.

Only data for the analyses of the intercellular atmosphere of Rome Beauty apples held under these tests are available, but they bear out the suggestion that the oxygen supply was very largely depleted in the coated fruit. These data are reported in Table VII.

From these results, which represent averages of a number of determinations, it is apparent that coating the surface of the fruit had so reduced available oxygen as to render anerobic respiration probable, whereas untreated fruit had an abundance of oxygen within the tissues. Data in Table VI showing a greater CO_2 output per unit O_2 intake in oil-coated than in untreated fruit indicate that anerobic respiration was already occurring to a limited extent.

This conclusion is further borne out by results reported in Table VIII, which records respiration data for Winesap and Rome Beauty apples held at a temperature of 80° F. (26.5° C).

TABLE VIII.—*Respiration of apples at 80° F. (26.5 C.)*

Experiment No.	Description of fruit.	Weight of fruit.	Run No.	Length of run.	Kilogram hours.	Weight of CO_2	CO_2 per kilogram hour.	CO_2 per kilogram hour.	O_2 per kilogram hour.	Respiratory ratio $\frac{CO_2}{O_2}$
		Gms.		*Hours.*		*Mgm.*	*Mgm.*	*Cc.*	*Cc.*	
1	Winesap; after 4 months at 32 F.; unwrapped box.	2,740	1	70	191.8	4,786.6	24.95	12.70	11.65	1.09
			2	69½	191.1	4,404.7	23.05	11.73	12.24	.96
2	Winesap; similar to experiment 1, except coated with mineral oil when removed to 26.5° C.	2,650	1	70	185.5	3,207.5	17.3	8.81	4.20	2.15
			2	69½	184.8	2,812.8	15.2	7.74	5.76	1.34
3	Winesap; same as experiment 1.	2,637	1	69½	184.1	4,470.8	24.3	12.37	11.43	1.08
			2	69	184.4	4,173.8	22.6	11.50	12.12	.95
4	Rome Beauty; early pick; after 4 months at 32° F.; no treatment.	3,113	1	69½	216.9	5,755.3	26.5	13.49	12.89	1.05
		3,005	2	69½	210.9	4,978.6	23.7	12.06	12.88	.94
5	Rome Beauty; similar to experiment 3, except oil-coated when removed to 26° C.	2,525	1	69½	176.1	3,563.9	20.2	11.20	4.57	2.45
			2	69½	176.3	2,937.6	16.7	8.50	7.15	1.19

Experiments 1 and 3 show normal Winesap apples under test at 80° F., with experiment 2 showing similar fruit oil coated when the test was started. Normal fruit has a respiratory ratio averaging 1.025 and 1.015 for experiments 1 and 3, respectively, while the oil-coated fruit gave a ratio of 1.75. Results on Rome Beauty were very similar. The rate of CO_2 evolution in both varieties was reduced from normal only about 35 per cent by the oil coating. This reduction was no greater in proportion than that at 64.5° (see Table VI). At the higher temperature, however, oxygen could not enter the oil-coated fruit sufficiently rapidly to supply the need, and anerobic respiration resulted. Analyses for the internal atmosphere of some of the fruits held at 80° F. are recorded in Table IX.

TABLE IX.—*Composition of intercellular atmosphere of apples held at 80° F. (26.5° C.)*

Respiration experiment No. (Table VIII).	Description of fruit.	CO_2.	O_2.	Per cent N_2 by difference.
		Per cent.	*Per cent.*	
1, 3	Winesap, untreated	9.2	14.2	76.6
2	Winesap, oil coated	14.0	1.7	84.3
4	Rome Beauty, untreated	5.5	16.2	78.3
5	Rome Beauty, oil coated	18.8	2.9	78.3

Analyses for oxygen in the fruit held at 80° F., where the respiratory ratio was much superior to unity, showed about the same quantity present as in similar fruit held at 64.5° F., where the anerobic respiration apparently was just starting, and apparently represents about the concentration present when oxygen is the limiting factor in fruit respiration.

The total quantity of oxygen entering the oil-coated fruit at 64.5° F. was approximately the same as the total amount entering at 80° F. It appears, therefore, that in fruit coated as heavily as the fruit here tested about 60° marked the highest temperature at which the oxygen supply entering the fruit was sufficient to prevent anerobic respiration. When held at temperatures above 60° anerobic respiration was very pronounced.

DISCUSSION OF EFFECT OF COATING SURFACE OF FRUIT UPON THE RIPENING PROCESSES

From the data presented above it is apparent that coating the surface of the fruit with such substances as oil or paraffin tends to retard respiration, regardless of the temperature at which the fruit is held, and thus to retard the ripening processes. The degree of this retardation will vary with the thickness of the coating. Apples heavily coated with either oil or paraffin are retarded in ripening much more than apples lightly coated.

So long as the substances used in coating the fruit are entirely tasteless and odorless, the flavor appears to be normal until anerobic respiration sets in. Anerobic respiration may occur in fruit held at 32° F., if the coating is very heavy. The higher the temperature at which the fruit is held the less the coating that will be required to induce anerobic respiration. At 64.5° fruit lightly coated appeared to show just the beginning of anerobic respiration, while the respiration of similarly treated fruit when held at 80° F. was largely anerobic. In fact it seems highly probable that anerobic respiration will occur in certain fruits at very high temperatures without any coating of the surface. Taylor and Overholser (*26*) reported that very high temperatures inhibited the ripening of Bartlett pears, with associated loss of quality. It seems probable that anerobic respiration occurred. Gerber's high respiratory ratio on apples held at very high temperatures (30° and 33° C.) may have been due to oxygen depletion in the tissues.

There is a wide variation in the amount of oxygen in the tissues of different varieties of apples under similar conditions. Magness and

Burroughs [9] found 5 to 6 per cent oxygen in western Winesap apples at 68° and 11 to 14 per cent in New York Baldwin apples under the same conditions. Table IX shows 14 per cent oxygen in Winesap apples grown in Virginia even at a temperature of 80°. Magness (*20*) found only 5.5 per cent oxygen in the tissues of California-grown Yellow Newtown apples at 68° F. (20° C.), while Virginia-grown Ben Davis showed 13 to 14 per cent oxygen at 80° F., and Delicious 16 to 17 per cent. Thus it is apparent that the internal atmosphere will vary with the skin permeability, which in turn will vary with the variety and the conditions under which it is grown.

The fact appears definitely established that coating the surface of the fruit, either with paraffin or with oil, will somewhat retard the ripening of the fruit. If too much is applied, however, anerobic respiration will result, and the flavor of the fruit will be injured. The amount that can be applied without injury will vary with the variety and with the temperature to which the fruit will be exposed. Very thin coatings may result in bad flavor in certain varieties, particularly when these varieties are exposed to the relatively high temperatures usually encountered by apples before ultimate consumption.

The effect of coating appears to be twofold. At certain temperatures there seems to be an actual limitation in the oxygen supply which not only retards ripening but also results in bad flavor. At all temperatures there is an apparent increase in the concentration of CO_2 within the tissues, and this increased concentration of CO_2 seems directly to inhibit the ripening processes, as will be discussed later.

INFLUENCE OF CARBON DIOXIDE CONCENTRATIONS UPON THE RIPENING OF APPLES

It has long been known that increasing the carbon dioxid pressure in the atmosphere surrounding plant tissues would have a marked effect upon their metabolism. Brooks, Cooley, and Fisher (*3*) reported that apples held in concentrations of 5 per cent and upward of CO_2 remained firm and green, but developed an alcoholic flavor. More recently, Kidd and West (*14*) have carried on extensive tests on storing apples in CO_2 with diminished O_2 pressures. Fruit has been stored in tight chambers and the chambers kept closed until the O_2 in the air was reduced to from 5 to 8 per cent, and the CO_2 reached 15 per cent, due to the respiration of the fruit. Then sufficient ventilation was given to maintain this ratio. They found it important not to reduce the O_2 pressure below 5 per cent or to increase the CO_2 above 15 per cent. They report that apples so handled keep about twice as long as controls held in air.

In order to determine the influence of CO_2 pressure, as distinct from limitation of O_2, upon the rate of ripening of apples, preliminary experiments were made with holding apples in various concentrations of CO_2. The apparatus used is shown in Figure 15. It was desirable not to keep the fruit in a closed chamber, where respiration from the fruit itself would alter the composition of the surrounding air. Consequently, two large bottles were used, A and B, each of 19 liters capacity. Bottle A was filled with water. In B the desired gas mixture was secured by introducing pure CO_2, O_2, and air in the desired amounts, using water displacement to measure the volumes. A layer of mineral oil was left in the bottom of this bottle to reduce unequal absorption of

[9] MAGNESS, J. R., and BURROUGHS, A. M. OP. CIT.

the gases by water. The bottle was closed with a stopper containing a tube (*t*) extending to the bottom of the bottle, and with another tube extending from the top of the bottle B to the desiccator C containing the apples under test. A siphon from A entered the top of the tube *t*, which delivered under the oil in B. As water flowed through this siphon into the bottom of bottle B, the contained gases passed out and through the desiccator C. They were delivered into the bottom of C, and escaped through a capillary opening in the top of C.

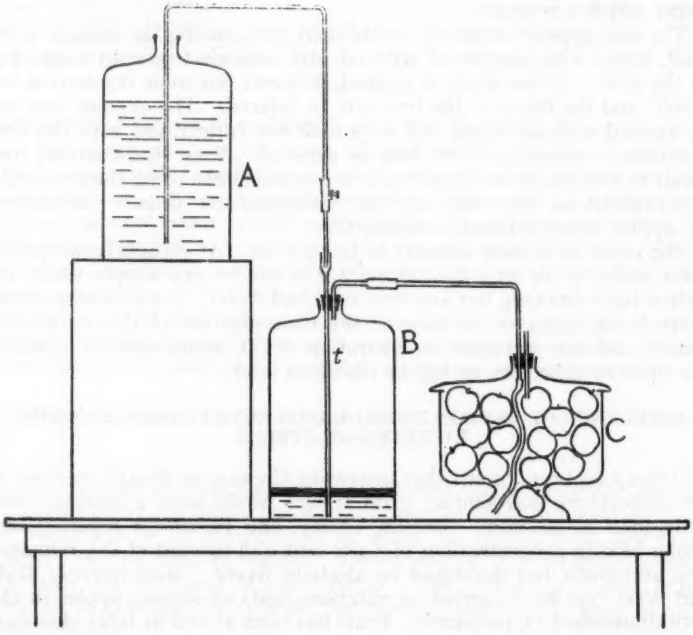


FIG. 15.—Apparatus for determining the influence of CO_2 pressure upon the rate of ripening of apples.

In order to determine whether or not any variation found was due to CO_2 present rather than to a deficiency of O_2, the O_2 content of the atmosphere in all tests was kept at approximately 20 per cent, or normal air concentration. CO_2 concentrations were varied from 0 to 50 per cent.

Some variations occurred in the rate of flow of the gas through the desiccators containing the apples. In all cases, however, about 18 liters of gas passed through per 24 hours, so there were never more than slight accumulations of CO_2 or deficiencies in O_2 due to the respiration of the fruit.

Two sets of experiments were run, one on Winesap and one on Delicious apples. Pressure tests, to determine the relative rates of softening, were made on a representative sample of the fruit at the beginning of the test and on all the various lots at the end of the test. Acidity determinations were also made on all lots. Data for Winesap apples are recorded in Table X.

TABLE X.—*Relative hardness and acidity in Winesap apples after being held 10 days at 71.5° F. (22° C.) in various concentrations of CO_2.*[a]

Description of test.			Pressure test.	Acidity—cc. N/10 acid per 10 gm. wet weight.
Lot No.	Per cent CO_2.	Per cent O_2.		
1	0	20	12.37	6.55
2	5	20	13.07	6.41
3	10	20	13.52	6.22
4	20	20	13.48	6.36
5	50	20	14.22	6.27
6	100	0	14.78	6.14

[a] Removed from 32° F. at beginning of test. Pressure test, 15.37 pounds. Acidity, 6.53 cc. N/10 per 10 gm. of wet tissue when removed from 32° F.

Internal atmospheres were analyzed from 5 apples of each lot at the end of the test. Oxygen present in all fruits (except lot No. 6, not analyzed) ranged from 6 to 13 per cent, while CO_2 ranged approximately 20 per cent above the concentration of the surrounding atmosphere. These analyses indicate that there was in all lots an abundance of O_2 within the tissue.

There was a marked relationship between CO_2 concentration and the rate of softening of the fruit. An atmosphere containing even 5 per cent CO_2 resulted in a distinctly slower rate of softening than did air of the normal concentration, while higher concentrations resulted in still further decreasing the softening rate. Acidity, on the other hand, apparently, disappeared more rapidly at the higher concentrations of CO_2. While the acidity results are all so close together that they are not at all conclusive, the uniformity of the fruit used in these tests as well as the regularity of the results makes them very suggestive. Results of a similar series of tests on Delicious apples are recorded in Table XI.

TABLE XI.—*Relative hardness and acidity in Delicious apples after being held 11 days at 71.5° F. (22° C.) in various concentrations of CO_2.*[a]

Description of test.			Pressure test.	Acidity—cc. N/10 acid per 10 gm. wet tissue.
Lot No.	Per cent CO_2.	Per cent O_2.		
			Pounds.	
1	0	20	11.78	2.26
2	5	20	12.31	2.43
3	10	20	12.63	2.31
4	20	20	13.82	2.21
5	50	20	13.58	2.26
6	100	0	13.58	2.22

[a] Removed from storage at 32° F. at beginning of test. Pressure test, 13.68 pounds. Acidity, 2.36 cc. N/10 acid per 10 grams of wet tissue when removed from storage at 32° F.

The decreased rate of softening of Delicious when held in various concentrations of CO_2 was even more marked than that for Winesap. There was little softening in any lot held in concentrations of CO_2 of 20 per cent or more. Results for acidity change are less pronounced. The results as a whole for Delicious are rather less dependable than those for Winesap because of the lack of uniformity in the samples used.

EFFECT OF CO_2 CONCENTRATION ON FLAVOR

A number of apples from each of the above tests were tested for flavor. Concentrations of 5 and 10 per cent CO_2 gave no flavor that was distinctive from that of control lots held in air. In the lot held in 20 per cent CO_2 most apples were normal, though in some fruit there was a suggestion of a flavor resulting from anerobic respiration. Apparently, 20 per cent CO_2 marked the extreme concentration in which apples can be held without serious impairment of flavor. Fruit held in 50 per cent CO_2 with 20 per cent O_2 as well as fruit held in 100 per cent CO_2 was entirely inedible. It is of much interest to note that the flavor produced in the apples in high CO_2 concentration, even in the presence of abundant O_2 in the tissues, is very similar to that produced by partially sealing the fruit with oil or paraffin, and in many fruits was very similar to the flavor of water-cored apples.

GENERAL DISCUSSION OF THE EFFECT OF COATING FRUIT, AND CO_2 CONCENTRATIONS, UPON THE RIPENING PROCESSES

It seems well established from the preceding data that coating the fruit with any material which retards gaseous exchanges will tend to retard softening and the development of a yellow color. This treatment results in a higher concentration of CO_2 within the tissues as well as a decreased O_2 supply. At lower temperatures, coating the fruit moderately does not result in a lack of oxygen within the tissues, but the higher concentration of CO_2 appears to directly retard the ripening processes. At higher temperatures both CO_2 accumulation and O_2 deficiency may take part in retarding the ripening process.

The fact that high concentrations of CO_2, even in the presence of oxygen, gives a flavor to the fruit similar to that resulting from an absence of O_2 suggests that the action of the CO_2 may be to inhibit the oxydizing enzymes, and thus to stop or retard normal oxidation.

INFLUENCE OF OILED PAPER WRAPS UPON RIPENING PROCESSES

There is a widespread belief in the commercial apple trade that wrapping the apples in oiled papers, as has been extensively done for the control of storage scald, results in a slower ripening of the fruit and a prolonging of the storage life (7). The above data on coating the fruit with oil or paraffin are very suggestive in this connection.

The papers that have been used for wrapping to control scald carry a very high oil content. The oil will leave the paper very readily, as may be shown by placing an oiled paper over one that contains no oil. The oil will quickly spread to the non-oiled paper. The waxy coating, or cuticle, of apples appears to be exceedingly soluble in mineral oil. Consequently, heavily oiled paper loses a portion of its oil to the fruit wax. It has been noted that where sufficient oil has been used to control scald there is often a distinct appearance of oiliness on the fruit surface.

While the oil coating thus secured would probably never be sufficient to result in bad flavor in the fruit, due to its inducing anerobic respiration, it undoubtedly differs in degree only from the effect produced by the heavier oil coating secured by wiping the fruit with a well oiled cloth, the method followed in the "oiled coating" here discussed. In the storage tests of holding fruit in oiled papers in connection with this work, small-sized wrappers were used, and there was apparently little, if any, oil transferred to the fruit. Under these circumstances no variation in rate of ripening in storage could be detected between fruit not wrapped and fruit wrapped in oiled paper. The wraps used, however, also failed to control the development of storage scald, although containing a very appreciable quantity of oil. The results obtained with this paper, together with the general appearance of fruit on which storage scald has been controlled by oiled wraps, strongly suggests that some oil must be transferred from the wrapper to the fruit if scald control is to be effective. In that case, the ripening of the fruit would be somewhat retarded by the use of oiled wraps.

SUMMARY

Changes in apples as they approach the condition of ripeness on the tree have been studied. These changes include (1) increase in size, (2) increase in the area and intensity of the red color and the change in the green or ground color from leaf green to yellow green, (3) a progressive softening of the fruit, and (4) a decrease in the apparent acidity in the fruit. Fruit softened very rapidly while still on the trees at Arlington, Va., during the warm, dry autumn of 1922.

Changes in the fruit following picking are primarily a continuation of pre-picking changes. Softening, acidity change, sugar change, etc., continue after picking much as while the fruit is still on the tree. The rate of these changes varies with the temperature at which the fruit is held.

Rate of softening of apples while in 32° F. storage as compared with storage at 70° F. varies with varieties. Ben Davis softened as much in 2½ months at 32° F. as in 12 days at 70° F., Winesap and Rome Beauty as much in 3 to 4 months at 32° F. as in 12 days at 70° F., York Imperial as much in about 5 months at 32° F. as in 12 days at 70° F., while Delicious and Rhode Island Greening were softer at the end of 12 days at 70° F. than they were at the end of their storage period, about 6 months, when placed in 32° storage immediately after picking.

All varieties used showed a constant decrease in acidity during the time the apples were held in 32° storage. Rate of decrease was very nearly the same in all varieties, regardless of acid content. Thus Delicious, with an initial acid content of about 3 cubic centimeters N/10 acid per 10 grams of wet tissue, lost acid until after 6 months only 2 cubic centimeter N/10 acid was present, a loss of from 30 to 40 per cent. Rhode Island Greening, with an acid content equivalent to 9 cubic centimeters N/10 acid at the beginning of the season, also lost only about 1 cubic centimeter N/10 acid, or about 12 per cent. Range of varieties tested, in order of decreasing acid content, is Rhode Island Greening, Ben Davis, York Imperial, Winesap, Rome Beauty, and Delicious. Percentage loss of acid during 6 months in storage is in the reverse order, those varieties showing lowest total acidity showing highest percentage loss.

Coating the surface of the fruit, either with paraffin or with oil, reduces the permeability of the fruit skin in proportion to the amount of the coating material applied. This results in higher CO_2 concentration, and lower O_2 concentration, in the gas present in the intercellular spaces of the fruit. If too much of the coating material is applied, anerobic respiration will result, with the development of disagreeable flavors in the fruit.

Increasing the concentration of CO_2 in the atmosphere surrounding apples results in a slower rate of softening of the apples. Coating the surface of the fruit with paraffin or oil also results in a slower rate of softening both in storage at 32° F. and at 70° F., probably by increasing the CO_2 concentration within the tissues.

Coating the fruit, either with paraffin or oil, resulted in a reduced respiration rate, whether the fruit was being tested at 32° F. (0° C.), at 64.5° F. (18° C.), or at 80° F. (26.5° C.); at 32° F. the ratio $\frac{CO_2}{O_2}$ was less than 1, indicating that there was an abundance of O_2 within the tissue, and that the reduced respiration rate was due to CO_2 accumulation. At 64.5° F., O_2 within the tissues was practically exhausted, and some anerobic respiration apparently occurred in coated fruit, for the respiratory ratio $\frac{CO_2}{O_2}$ was greater than 1. At 80° F. the ratio $\frac{CO_2}{O_2}$ was much greater than 1, indicating marked anerobic respiration.

In normal, uncoated fruit the respiratory ratio at all temperatures was approximately 1, indicating that there was no anerobic respiration in any of the normal fruit studied. Rate of CO_2 evolution in normal Winesap apples averaged 1.97 milligrams per kilogram hour at 0° C., 16.45 milligrams at 18° C., and 23.73 milligrams at 26.5° C. This appears to be very close to the ratio of the softening rates at the different temperatures.

Atmospheres of CO_2 in concentrations of 5 per cent, 10 per cent, 20 per cent, and 50 per cent, with 20 per cent O_2, markedly inhibited the softening rates of apples, the retardation in softening rate varying with the CO_2 concentration. Five per cent and 10 per cent concentrations of CO_2 had no appreciable effect upon the flavor of apples. In concentrations of 20 per cent CO_2 there was a very slight flavor of fermentation. Fruit held in 50 per cent CO_2 was entirely inedible. These tests were all made at 71.5° F. (22° C.). They indicate that any concentration of CO_2 in which men can work, such as the concentrations of 2 to 3 per cent which may occasionally occur in cold-storage rooms, will not injure the fruit, and may, in fact, be distinctly beneficial, through retarding the softening process.

LITERATURE CITED

(1) Ballard, W. S., Magness, J. R., and Hawkins, L. A.
1922. Internal browning of the Yellow Newtown apple. U. S. Dept. Agr. Bul. 1104, 24 p., 2 col. pl.

(2) Bigelow, W. D., Gore, H. C., and Howard, B. J.
1905. Studies on apples. U. S. Dept. Agr. Bur. Chem. Bul. 94, 100 p., 30 fig., 5 pl. Bibliographical footnotes.

(3) Brooks, Charles, Cooley, J. S., and Fisher, D. F.
1919. Apple-scald. *In* Jour. Agr. Research, v. 16, p. 195–217, 11 fig.

(4) Browne, C. A., Jr.
1899. A chemical study of the apple and its products. Pa. Dept. Agr. Bul. 58 46 p.

(5) BURROUGHS, A. M.
1923. CHANGES IN THE RESPIRATION RATE OF RIPENING APPLES. *In* Proc. Amer. Soc. Hort. Sci., 1922, p. 225–235.
(6) CLARK, W. Blair.
1917. A SAMPLING PRESS. *In* Jour. Indus. and Engin. Chem., v. 9, p. 788–790.
(7) CLAWSON, O. T.
1923. WENATCHEE SHIPPERS SOLD ON OIL WRAPS. *In* Wenatchee Fruit Grower, v. 2, no. 3, p. 6, 10, 32, illus.
(8) CORBETT, L. C.
1917. COLOR AS AN INDICATION OF THE PICKING MATURITY OF FRUITS AND VEGETABLES. *In* U. S. Dept. Agr. Yearbook, 1916, p. 99–106, pl. A–F (col.)
(9) CUMMINGS, M. B., and LOMBARD, P. M.
1915. FARM APPLE STORAGE. III. STRUCTURAL CHARACTERS IN RELATION TO STORAGE. *In* Vt. Agr. Exp. Sta. Bul. 186, p. 130–136, 5 fig., 2 pl.
(10) DIEHL, H. C., and MAGNESS, J. R.
1922. THE RIPENING OF CALIFORNIA PLUMS. *In* Mo. Bul. Calif. Dept. Agr., v. 11, p. 387–392.
(11) EHRLICH, Felix.
1917. DIE PEKTINSTOFFE, IHRE KONSTITUTION UND BEDEUTUNG. *In* Chem. Ztg., Jahrg. 41, p. 197–200.
(12) GERBER, C.
1896. RECHERCHES SUR LA MATURATION DES FRUITS CHARNUS. *In* Ann. Sci. Nat. Bot., sér. 8, t. 4, p. 1–280, 2 pl.
(13) GORE, H. C.
1911. STUDIES ON FRUIT RESPIRATION. U. S. Dept. Agr. Bur. Chem. Bul. 142, 40 p., 17 fig.
(14) GREAT BRITAIN. Food Investigation Board.
1922. REPORT OF FRUIT AND VEGETABLES COMMITTEE. *In* Rpt. Food Investigation Bd., 1921, p. 14–28, 4 pl.
(15) HAWKINS, Lon A.
1921. A PHYSIOLOGICAL STUDY OF GRAPEFRUIT RIPENING AND STORAGE. *In* Jour. Agr. Research, v. 22, p. 263–279, 1 fig. Literature cited, p. 278–279.
(16) ——— and MAGNESS, J. R.
1920. SOME CHANGES IN FLORIDA GRAPEFRUIT IN STORAGE. *In* Jour. Agr. Research, v. 20, p. 357–373. Literature cited, p. 372–373.
(17) HILL, George R.
1913. RESPIRATION OF FRUITS AND GROWING PLANT TISSUES IN CERTAIN GASES, WITH REFERENCE TO VENTILATION AND FRUIT STORAGE. N. Y. Cornell Agr. Exp. Sta. Bul. 330, p. 377–408. Bibliography, p. 407–408.
(18) LEWIS, C. I., MURNEEK, A. E., and CATE, C. C.
1919. PEAR HARVESTING AND STORAGE INVESTIGATIONS IN ROGUE RIVER VALLEY. Oreg. Agr. Exp. Sta. Bul. 162, 39 p., 12 fig.
(19) MAGNESS, J. R.
1920. INVESTIGATIONS IN THE RIPENING AND STORAGE OF BARTLETT PEARS. *In* Jour. Agr. Research, v. 19, p. 473–500. Literature cited, p. 499–500.
(20) ———
1920. COMPOSITION OF GASES IN INTERCELLULAR SPACES OF APPLES AND POTATOES. *In* Bot. Gaz., v. 70, p. 308–316, 1 fig. Literature cited, p. 316.
(21) MURNEEK, A. E.
1921. A NEW TEST FOR MATURITY OF THE PEAR. (Third report.) Oreg. Agr. Exp. Sta. Bul. 186, 28 p., illus.
(22) OTTO, R.
1902. UEBER DAS SCHWITZENLASSEN DER AEPFEL. *In* Proskau. Obstbau. Ztg., Jahrg. 7, p. 156–159.
(23) PERRY, Winifred, and MARTIN, J. N.
1918. THE CUTINIZATION OF APPLE SKINS IN RELATION TO THEIR KEEPING QUALITIES AND THEIR ENVIRONMENT. *In* Rpt. Iowa State Hort. Soc., v. 52 (1917), p. 180–192, illus.
(24) RAMSEY, H. J., MCKAY, A. W., MARKELL, E. L., and BIRD, H. S.
1917. THE HANDLING AND STORAGE OF APPLES IN THE PACIFIC NORTHWEST. U. S. Dept. Agr. Bul. 587, 32 p., 7 pl.
(25) SCHRYVER, S. B., and HAYNES, Dorothy.
1916. THE PECTIC SUBSTANCES OF PLANTS. *In* Biochem. Jour., v. 10, p. 539–547.

(26) TAYLOR, R. H., and OVERHOLSER, E. L.
1919. SOME EFFECTS OF HIGH TEMPERATURE AND HUMIDITY UPON THE KEEPING QUALITY OF BARTLETT PEARS. *In* Mo. Bul. State Com. Hort. [Calif.] v. 8, p. 118–125.

(27) WHITEHOUSE, W. E.
1916. A STUDY OF VARIATION IN APPLES DURING THE GROWING SEASON. Oreg. Agr. Exp. Sta. Bul. 134, 13 p.

(28) ZSCHOKKE, A.
1897. UEBER DEN BAU DER HAUT UND DIE URSACHEN DER VERSCHIEDENEN HALTBARKEIT UNSERER KERNOBSTFRÜCHTE. *In* Landw. Jahrb. Schweiz, Bd. 11, p. 153–197, 2 pl. Bibliographical footnotes.

A STUDY OF THE EFFECTS OF PUMPKIN SEEDS ON THE GROWTH OF RATS[1]

By BENJAMIN MASUROVSKY[2]

Animal Nutrition Laboratory, Dairy Husbandry Department, Nebraska Agricultural Experiment Station

INTRODUCTION

The object of the experiment recorded in this paper was to investigate the belief prevalent among stock feeders as to the injury caused by of *Cucurbita pepo* (pumpkin) seeds in animal metabolism and to verify the results obtained by scientific investigators in the study of the effects of these seeds upon animals.

Pott [3] states that the claim made that pumpkin seeds are injurious is without foundation.

Lindsey [4] found that the seeds appeared to have no injurious effects upon animals when fed in the amounts found in the entire pumpkin fruit.

The writer [5] fed pumpkins with increased quantities of Cucurbita seeds to growing pigs and found no detrimental effect upon the gain in body weight of the animals. Elsewhere he [6] experimented on himself by including *Cucurbita pepo* seeds in his diet. In studying the kidney excretion he found that the rôle played by pumpkin seeds in animal metabolism is of a chemico-pharmacognostic value.

EXPERIMENT

Six rats of the same litter resulting from the crossing of a pure Norwegian buck and an albino doe of the "Tyler" strain were used in this experiment. The foundation stocks of both the Norwegian and albino strains were purchased from the Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

The experimental animals were born April 24, 1923, and weaned May 18. They were all males and at the beginning of the feeding experiment, June 22, were 60 days old and of the following weights:

No. of group.	No. of animal.	Sex.	Weight.
			Gm.
I	41	♂	135
	43	♂	137
II	45	♂	125
	46	♂	127
III	40	♂	118
	42	♂	134

[1] Accepted for publication Nov. 1, 1923. Published by permission of the Director, Nebraska Experiment Station.

[2] The writer wishes to express his thanks to R. A. Braun, Dairy Husbandry Department, for the timely help given in this work.

[3] POTT, Emil. HANDBUCH DER THIRISCHEN ERNÄHRUNG UND DER LANDWIRTSCHAFTLICHEN FUTTERMITTEL. Bd. 2, p. 424-425. 1907.

[4] LINDSEY, J. B. THE COMPOSITION, DIGESTIBILITY, AND FEEDING VALUE OF PUMPKINS. Mass. Agr. Exp. Sta. Bul. 174, p. 55-71. 1917.

[5] MASUROVSKY, Benjamin. EFFECTS OF SOME CUCURBITA SEEDS ON ANIMAL METABOLISM. *In* Jour. Agr. Research, v. 21, p. 523-539. 1921.

[6] MASUROVSKY, Benjamin. A STUDY OF THE EFFECTS OF CUCURBITA PEPO SEEDS ON KIDNEY EXCRETION. *In* Proc. Nat. Acad. Sci., v. 8, p. 39-43. 1922.

The balanced basal ration consisted of the following ingredients:

	Gm.		Gm.
Wheat	2,900	Meat scraps	1,000
Corn	2,800	Cod liver oil	300
Oats (rolled)	750	Calcium carbonate	150
Green peas	750	Sodium chlorid	100
Alfalfa (flour)	500		

The pumpkin seeds used in this experiment were obtained from a seed store. All the grain was milled very fine and thoroughly mixed.

Up to June 22 all experimental animals were kept on the same basal balanced ration, and beginning with June 1 their gain in weight was recorded weekly. On June 22 the animals were arranged for the feeding trial in the following way: Three groups of rats, two animals in each group, were placed in three separate cages and fed on different grain mixtures. Group I, No. 41 and 43, were fed on the basal balanced ration and served as a control in this experiment; Group II, No. 45 and 46, received a grain mixture consisting of 50 parts ground pumpkin seeds and 50 parts of the basal balanced ration; Group III, No. 41 and 42, were fed ground pumpkin seeds only. The food was given without restriction and a constant supply of fresh tap water was kept before the animals.

The all-wire cages were provided with false bottoms wherein a thin layer of wooden shaving served as bedding for the animals. The litter was removed weekly, the false bottoms disinfected, and clean bedding was provided for the animals. Weekly records of body weight of the rats were kept until the completion of the feeding trial. The results are given in the accompanying tables:

TABLE I.—*Pumpkin seed experiment*

No. of group.	No. of animal.	Initial weight.	June 8 weight.	Total gain per 100 gm.	Daily gain per 100 gm.	June 16 weight.	Total gain per 100 gm.	Daily gain per 100 gm. live weight.	June 22 weight.	Total gain per 100 gm.	Daily gain per 100 gm.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I [a]	41	68	88	.....	.....	117	.....	.....	135	.....	.....
	43	67	87	+29.5	+4.22	118	+34.2	+4.27	137	+15.7	+2.61
II [b]	45	65	85	.....	.....	110	.....	.....	125	.....	.....
	46	73	89	+26.0	+3.71	113	+28.1	+3.51	127	+13.0	+2.16
III [c]	40	66	86	.....	.....	106	.....	.....	118	.....	.....
	42	68	88	+26.8	+3.82	118	+31.7	+3.96	134	+12.5	+2.08

[a] Control; fed balanced ration.
[b] Fed 50 parts pumpkin seed and 50 parts balanced ration.
[c] Fed pumpkin seeds only.

TABLE I.—*Pumpkin seed experiment*—Continued

No. of group.	No. of animal.	Initial weight.	June 30 weight.	Total gain per 100 gm.	Daily gain per 100 gm. live weight.	July 16 weight.	Total gain per 100 gm. live weight.	Daily gain per 100 gm. live weight.	July 24 weight.	Total gain per 100 gm.	Daily gain per 100 gm. live weight.	July 30 weight.	Total gain per 100 gm. live weight.	Daily gain per 100 gm. live weight.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I [a]	41	135	168	.....	.....	195	.....	.....	215	.....	.....	234	.....	.....
	43	137	270	+24.2	+3.02	200	+16.8	+2.8	235	+13.9	+1.73	263	+10.4	+1.73
II [b]	45	125	272	.....	.....	192	.....	.....	220	.....	.....	245	.....	.....
	46	127	273	+36.9	+4.60	195	+12.17	+2.03	223	+14.4	+1.8	242	+9.93	+1.65
III [c]	40	118	249	.....	.....	171	.....	.....	190	.....	.....	197	.....	.....
	42	134	359	+22.2	+2.77	182	+14.6	+2.43	200	+10.4	+1.3	213	+5.12	+0.85

No. of group.	No. of animal.	Initial weight.	July 27 weight.	Total gain per 100 gm.	Daily gain per 100 gm.	Aug. 3 weight.	Total gain per 100 gm.	Daily gain per 100 gm.	Aug. 10 weight.	Total gain per 100 gm.	Daily gain per 100 gm.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I [a]	41	234	251	.....	.....	270	.....	.....	288	.....	.....
	43	263	285	+7.84	+1.12	310	+8.20	+1.17	327	+6.03	+0.89
II [b]	45	245	275	.....	.....	300	.....	.....	317	.....	.....
	46	242	260	+9.85	+1.40	278	+8.03	+1.14	297	+6.22	+0.88
III [c]	40	197	183	.....	.....	200	.....	.....	208	.....	.....
	42	213	220	−1.70	−0.24	216	+3.22	+0.46	220	+2.88	+0.41

[a] Control; fed balanced ration.
[b] Fed 50 parts pumpkin seeds and 50 parts balanced ration.
[c] Fed pumpkin seeds only.

DISCUSSION

From the tables here presented it will be found that calculations were made by subtracting the weekly weight of each animal from the

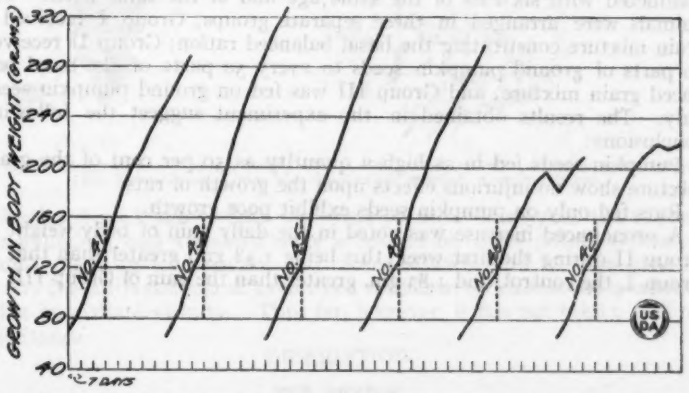


FIG. 1.—No. 41 and 43 belong to Group I, which were fed the basal balanced ration. No. 45 and 46 belong to Group II and received 50 parts of ground pumpkin seeds for every 50 parts of the basal balanced ration. No. 40 and 42 constitute Group III, which were fed ground pumpkin seeds only. The curves represent the growth in body weight of the rats beginning June 1 and ending August 10. The broken lines pass through the points at which the feeding trial of pumpkin seeds started.

preceding weekly weight of the same animal. The total gain constituted the algebraic sum of the differences of all the animals in each group. The total gain per 100 gm. body weight was obtained by divid-

ing the total weekly gain by the total body weight of each group of the preceding week and multiplying the quotient by 100. The daily gain per 100 gm. body weight was derived by dividing the total weekly gain per 100 gm. body weight by the number of days in the week.

In studying the figures and the records given above it will be noticed that the change of environment and ration shortly after grouping of the experimental animals was most pronounced in Group II, i. e., on June 30 the daily gain per 100 gm. body weight for Group II was 1.83 gm. greater than that of Group III and 1.58 gm. greater than that of the control, Group I.

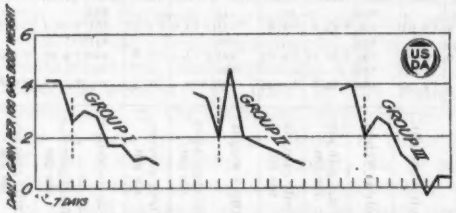


FIG. 2.—The curves represent the daily gain per 100 gm. body weight of each group of rats beginning June 1 and ending August 10. The broken lines pass through the points at which the feeding trial of pumpkin seeds started.

As will be noted from Figure 1, the animals of Group II show smooth growth curves similar to those of the control, while the animals in Group III depart considerably, a break being noticeable in their growth curves when compared either with those of Group I or Group II.

Figure 2 shows the stimulating effect of pumpkin seeds when mixed with the basal ration, 50 per cent of each, as fed to Group II. It also demonstrates the poor effect of pumpkin seeds, as the sole food, on gain in weight of growing rats.

SUMMARY

A seven-weeks feeding-trial of pumpkin seeds (*Cucurbita pepo*) was conducted with six rats of the same age and of the same litter. The animals were arranged in three separate groups; Group I received a grain mixture constituting the basal balanced ration; Group II received 50 parts of ground pumpkin seeds to every 50 parts of the basal balanced grain mixture; and Group III was fed on ground pumpkin seeds only. The results obtained in the experiment suggest the following conclusions:

Pumpkin seeds fed in as high a quantity as 50 per cent of the grain mixture show no injurious effects upon the growth of rats.

Rats fed only on pumpkin seeds exhibit poor growth.

A pronounced increase was noted in the daily gain of body weight in Group II during the first week, this being 1.58 gm. greater than that of Group I, the control, and 1.83 gm. greater than the gain of Group III.

THE ARGUS TORTOISE BEETLE[1]

By F. H. CHITTENDEN

Entomologist, Truck-Crop Insect Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTORY

The foliage of sweet potato, wherever planted in the United States, is attacked every year by tortoise beetles of different species, the largest of which is known as the Argus tortoise beetle (*Chelymorpha cassidea* Fab.).[2] This species breeds on convolvulaceous plants, and, until the year 1919, was rather generally believed to be more commonly found on bindweed (*Convolvulus* spp.) or wild morning-glory (*Ipomoea* spp.)

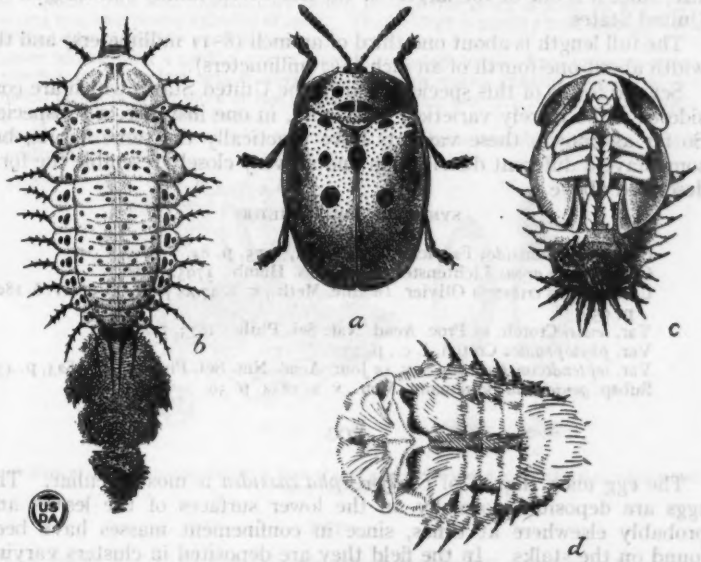


FIG. 1.—The Argus tortoise beetle: *a*, Beetle; *b*, larva with feci-fork extended at end; *c*, pupa, ventral view; *d*, dorsal view of pupa with characteristic covering. Greatly enlarged.

and related wild plants than on sweet potato. That year the species was abundant and attracted more attention on sweet potato (*Ipomoea batatas*) than on wild plants, and recent studies tend to show that it prefers the cultivated plant, even when wild Convolvulaceae are available in the immediate vicinity. Thus far, however, it has not been a pest of importance.

DESCRIPTION

THE BEETLE

The beetle (fig. 1, *a*, and Pl. 1, A, *a*) is dark brick red when fully mature, and, before it is fully colored, of different shades of yellow. Its

[1] Accepted for publication, Nov. 1, 1923.

[2] Formerly known as *Chelymorpha argus* Licht.; order Coleoptera, family Chrysomelidae.

upper surface is ornamented with black dots of variable number and size, the variation being largely dependent on locality. Usually, in the eastern form, there are from 17 to 21 spots, which are more or less rounded. On the prothorax there are usually from 4 to 6 spots, and there is also one sutural spot behind the scutellum. The small marginal spot is sometimes lacking, especially in western forms. The lower surface is black, with the exception of the head, margins of the prothorax, and that portion of the elytra which can be seen from below. The antennæ and legs are also black.

This form can readily be distinguished from all other genera of tortoise beetles by the characters given, and from other common species that attack sweet potato it can be known at once by its much larger size, since it is one of the largest of the leaf-beetle family occurring in the United States.

The full length is about one-third of an inch (8–11 millimeters) and the width about one-fourth of an inch (6–7 millimeters).

Several forms of this species occur in the United States which are considered to be merely varieties, races, and, in one instance, a subspecies. So far as known, these variants have practically the same habits, but some have a different distribution and all very closely resemble the form described above.

SYNONYMS AND VARIETIES

Chelymorpha cassidea Fabricius, Syst. Ent., 1775, p. 82.
Chelymorpha argus Lichtenstein, Cat. Mus. Hamb., 1795, p. 66.
Chelymorpha cribraria Olivier, *In* Enc. Meth., v. 5, 1790, p. 383; Ent., v. 6, 1808, p. 956.
Var. *lewisi* Crotch, *in* Proc. Acad. Nat. Sci. Phila., 1873, p. 77.
Var. *phytophagica* Crotch, l. c., p. 77.
Var. *septendecim-punctata* Say, *in* Jour. Acad. Nat. Sci. Phila., v. 3, 1823, p. 435.
Subsp. *geniculata* Bohemann, Mon., v. 2, 1854, p. 39.

EGG

The egg mass (fig. 2) of *Chelymorpha cassidea* is most peculiar. The eggs are deposited normally on the lower surfaces of the leaves and probably elsewhere at times, since in confinement masses have been found on the stalks. In the field they are deposited in clusters varying from 16 to 28. Six masses contained 16, 17, 18, 24, 26, and 28 eggs, respectively. The eggs are attached to the leaf by long pedicels, the pedicels being fastened to the leaf surface by a considerable amount of glutinous substance. The eggs also adhere to each other at their bases and, in many cases, halfway or a little more toward the apices, but the ends are free and divergent. The eggs are deposited irregularly, without definite pattern. There is usually a central irregular row of 6 eggs, flanked at each side by a similar irregular row of 5 or 6, while the outer rows together form a mass, which is always irregular, but with a suggestion of a circular arrangement.

The individual egg measures about 1.6 millimeters in length and 0.8 millimeter in width, being approximately twice as long as wide. It is evenly rounded at the base and bears at the distal end a cap which opens at one side when the larva issues and which bears at the extreme apex a prominent dark reddish tubercle of irregular shape, somewhat resembling a bit of sealing wax. The general color of the egg is dull buff and the surface is granulated.

LARVA

The larva (fig. 1, *b;* Pl. 1, A, *b;* B) may be described as follows:

Head prominent, dark brown, outline of basal half semicircular; eyes small, black; mandibles prominent, darker brown, width of head about 1.6 millimeters; surface with numerous short bristles. Thoracic plate nearly twice as long as wide, each half irregularly pentagonal. Body dull light yellow, strongly marked with numerous dark brown, nearly tuberculate spots; its form, including thorax, robust, less than twice as long as wide, somewhat depressed, armed with long prominent lateral spines, 14 on each side as follows: 4 thoracic, first pair directed forward and upward above head, second pair at acute angles and semierect and two pairs at right angles; 8 abdominal spines, slightly curving upward at apex, 2 anal spines erect. Each spine wide at base and light colored in basal half with strong lateral spines, apical half acuminate, black. Dorsum of abdominal segments each with two rows of transversely rounded oblong tubercles, those of first three segments largest, size diminishing posteriorly. Anal segment with well defined dark brown plate about twice as long as wide, terminating in a long proleg rounded at apex. The feci-fork is usually held slanting back from the body and rarely vertical or over it.

Lower surface with a median row of 5 small, rounded, longitudinal brown spots on segments 2 to 6, 6 to last with transverse dark-brown marks, growing stronger to last segment, posterior third more or less marked with dark brown. Legs long and stout, dark brown, blackish when folded.

Length of full-grown larva without feci-fork 7 millimeters (about ¼ inch), width 4.5 millimeters.

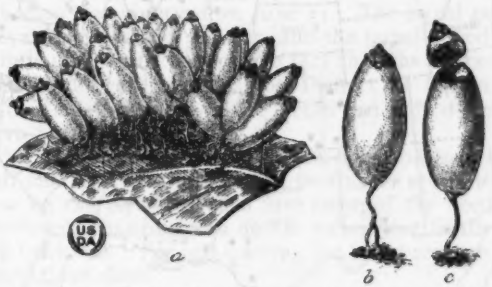


FIG. 2.—Eggs of the argus tortoise beetle: *a*, Mass of eggs, attached to leaf; *b*, *c*, individual eggs before and after hatching.

PUPA

The pupa (fig. 1, *c*, *d;* Pl. 1, A, *c*) is pale yellow, marked with dark brown, becoming nearly black toward the time of transformation to adult. The surface is nearly covered with a pale bluish bloom or waxy secretion resembling a mold, a peculiar covering not often seen in any other group of beetles. The ventral surface is somewhat flattened and the dorsal surface is convex. The thorax projects strongly at each side, being a little wider than the widest abdominal segment. It is armed apically with two short spinous processes in the proximal third. The antennal sheaths and legs are robust, the posterior pair being about as long as the elytral sheaths. The body is armed on each side with five long and strong black-tipped spines similar to those of the larva, the first pair situated about the middle of the body. The first two pairs are subequal in length and the remaining pairs decrease in length posteriorly. There are also two pairs of short unicolorous spines toward the apex. The cast skin of the larva with its spines is rolled up in a mass at the posterior extremity.

Length 8 millimeters, width 4.5 millimeters.

DISTRIBUTION

The Argus tortoise beetle is a native species inhabiting a large portion of the United States. It also occurs in Canada. The species does not appear to be recorded from Mexico, but several related species occur there. The known distribution, including what at present are considered varieties, is shown in Figure 3.

NOTES ON HABITS AND DEVELOPMENT

From larvae collected at Arlington, Va., June 20, 1919, and later, the first adults began to emerge June 29, continuing to emerge until July 3. In outdoor rearing cages emergence was from September 10 to the first week of October. Adults observed on sweet potato all developed within 7 to 10 days of each other. Individuals collected by Miss Marion T. Van Horn on wild bindweed (*Convolvulus* sp.) that grew in a shady location in the District of Columbia were, evidently as a result of not being exposed to direct sunlight, over two weeks late in development, while larvæ one-third to two-thirds grown were observed after the Arlington material, which was almost constantly exposed to sunlight, had all transformed to pupæ.

In 1919, 50 reared beetles were under observation during July. Of this number 20 were placed on growing sweet potato plants and covered

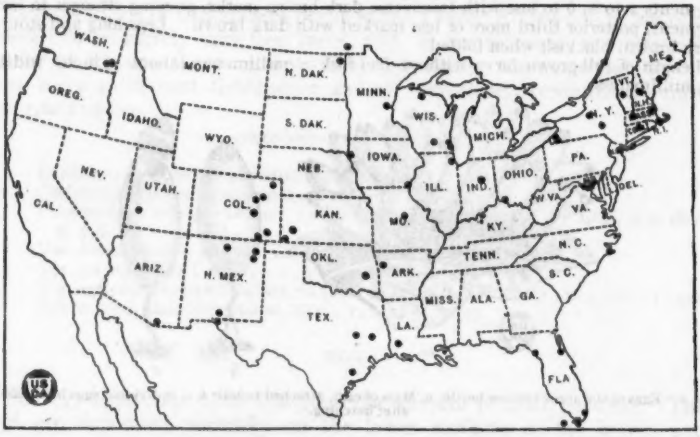


FIG. 3.—Map showing known distribution of the Argus tortoise beetle.

with a large cloth-covered rearing cage, but they did not thrive, some individuals dying, and no eggs could be found up to the end of the month, although in a second lot kept in the insectary eggs were observed August 3. Finally the cage was removed and the beetles allowed to shift for themselves. September 2 an egg mass was found on the same plat where the beetles had been feeding.

The experience of two years shows definitely that the second generation is only a partial one, since only three egg masses of this generation were found and at intervals of a month, indicating that the majority of the beetles of the first new generation hibernate, in this respect agreeing with some other insects.

Of the second generation, the eggs of which hatched during August, several pupæ were formed a month later, showing a larval period of about three weeks in rather cool summer weather.

In its apparently irregular development, *Chelymorpha cassidea* resembles to some extent the Colorado potato beetle. The overwintered beetles first occur some time in May—in 1920, May 17, in an exception-

ally cool spring. The first egg masses were obtained May 23. The first new generation develops during the last week of June and throughout July, with the temperature 60° to 100° F., averaging 75°.

May 17, 1920, adults were collected on the western edge of the truck farm at Arlington, Va., and on the far side of a large sewer pipe which had undoubtedly attracted them, as it conserved considerable heat. Nearly all of these beetles were on the west side, morning-glory plants which were numerous on the east side harboring only one specimen. The beetles hibernated on the more protected side and mostly together, since all specimens were found in two small areas, representing colonies, quite close to each other. Additional evidence of the eminently gregarious habit of this species was afforded the following morning by examination of the jar in which these beetles were placed overnight. At first glance it was thought that some had escaped, but close examination showed that they were closely huddled together in the folds of the small leaves.

Larvæ obtained in late August transformed to pupæ September 1, and the adults emerged September 10, which gives 9 days for the pupal period during moderately cool weather, with the temperature ranging from 70° to 82° F., and averaging 74° F.

The first pupa was observed on June 23. The larval period is about three weeks in cool summer weather, and the pupal period for the same temperature is about nine days. The fact that the species has only an exceptional second generation in the District of Columbia tends to show that it is single-brooded in the North and fully double-brooded in the Southern States.

The adults issue at any time during the day, and the coloring begins at the head and legs, the dots on the prothorax appearing some time before those on the elytra. When first emerged the beetles are bright yellow, afterwards changing to a darker yellow and finally to yellowish red or dark brick red. For full coloring the insect requires at least two, and probably three days.

The beetles cling most tenaciously to foliage or to objects in rearing cages, unless they drop down to "play 'possum," and evidently for that reason do not very often find their way into the collecting net, most individuals having been collected on their food plants and elsewhere, where attention was attracted to them because of their conspicuous coloring.

The first eggs obtained during 1920 were laid during the last week of May, beginning May 22, and the first larvæ were noticed June 10. Eggs that were laid June 4 and were isolated, hatched June 14, 10 days later. From this lot the larvæ began to transform to pupæ July 3 and the adults began issuing July 10. The pupæ began to transform to beetles at the same date in different jars kept under different atmospheric conditions.

The foregoing data furnish the following as an approximate average life cycle from egg to adult for the District of Columbia and vicinity:

PERIODS OF THE STAGES OF CHELYMORPHA CASSIDEA FAB.

	Days
Egg period:	
June 1 to June 11	10
June 4 to June 14	10
Larval period: June 14 to July 3	19
Pupal period: July 3 to July 10, September 1 to 10	7 to 9
Total from egg to adult	36

HISTORY AND LITERATURE

The Argus tortoise beetle was described, under the name *Coccinella cassidea*, by Fabricius in 1775 (*3*[3], *p. 82*).

In 1869, A. S. Packard (*10, p. 504*) figured the pupa and adult, with the statement that he had found all stages on the leaves of "silkweed" in July and early in August, and that in one instance in Salem, Mass., it occurred in abundance on raspberry.

In 1870, Riley (*11, p. 58; 12, p. 4*) published a short note on the larva and adult, stating that it was found on Asclepias.

In 1879, Harrington (*2, p. 120*) published a short note in which the species was reported as a new foe by market gardeners in Canada, where it occurred in immense numbers and destroyed plants and flowers. It was said to be first noticed on wild Convolvulus.

In the early eighties the writer received from Ovid, N. Y., specimens of the larva of the Argus tortoise beetle on raspberry.

The first record of the Bureau of Entomology is dated March 11, 1884, when specimens were received which were taken on the foliage of sweet potato at Touch Key, Fla.

June 28, 1886, Dr. J. M. Shaffer, Keokuk, Iowa, reported numbers of larvæ on sweet potato vines.

In 1887, Lintner (*8, p. 673*) wrote of this insect under the title "Milkweed beetle with bad habits." A correspondent in Chenango County, N. Y., stated that it was found on morning-glory, corn, cabbage, and plantain. In Doctor Lintner's reply he mentions other alleged food plants, including milkweed, mustard, and plants of the rose family.

In 1889, J. B. Wielandy reported the species on Convolvulus at Springer, N. Mex., on July 13, and on June 30 attack on sweet potato was reported at Slatonville, Ark., by D. D. Forman.

In 1893, Webster (*14, p. 204*) treated this insect as occurring on raspberry and blackberry.

In 1897, the writer published what appears to be the first account of attack by this species on sweet potato (*2, p. 23*). In 1898, Webster and Mally (*15, p. 99*) mentioned its occurrence at Willard, Ohio, on strawberry vines. In 1899 Lugger (*9, p. 254*) mentioned this insect as frequently being found on raspberry. In 1899, also, Sanderson (*13, p. 140–142*) gave a biological account of this species, with breeding records showing variability in the adults from the same mass of eggs. He also described the eggs, larvæ, and pupæ.

July 31, 1901, specimens were received from J. P. Reynolds, North Haven, Me., taken on rose leaves.

In 1905, Forbes (*5, p. 192*) included this species in a list of insects found on corn.

July 8, 1906, specimens were received from Silver Creek, N. Y., found on timothy, evidently an accidental occurrence. The same comment applies to specimens found next year in a cornfield at Arlington, Va. July 19, 1907, larvæ were reported by I. J. Condit attacking sweet potato at Benning, D. C., and in that year Fall and Cockerell (*4, p. 200*) mentioned its occurrence on Solanum in New Mexico.

August 15, 1908, Otis Andrews, El Paso, Tex., reported injury to morning-glory and moonflower. July 22, 1909, a canning company at Cherry Creek, N. Y., sent specimens of pea vines on which were found

[3] Reference is made by number (italic) to "Literature cited," p. 50–51.

many larvæ and adults of this tortoise beetle. The vines were also well covered with pupæ from which the adults were beginning to issue. July 28, Miss Julia D. Whiting, Deerfield, Mass., sent specimens found on morning-glory, including larvæ with the nymphs of the pentatomid predacious enemy *Apateticus bracteatus* Fitch. The same year Frederick Knab (*7, p. 152*) wrote a short note on the nuptial colors of this species.

July 15, 1910, report was received from F. H. Horsford that the pupa was found on the lower side of the leaves of *Lilium henryi* at Charlotte, Vt.

July 13, 1911, Fabian Garcia, Agricultural College, N. Mex., reported attack on sweet potato. July 2, 1913, F. B. Milliken collected this species at Garden City, Kans., on bush morning-glory (*Ipomoea leptophylla*).

In 1916, H. S. Barber (*1, p. 119*) included this species in a review of North American tortoise beetles, summing up briefly the habits of the species as published and furnishing a map showing its distribution.

May 16, 1917, J. A. Hanchey, Allen Parish, La., reported attack on sweet potato, the plants looking as though fire had gone through them. This, however, was only in spots and did not extend through entire fields.

During 1919 this insect, as previously stated, attracted much attention. It was first noticed attacking sweet potato at Arlington, Va., when full grown larvæ were observed. June 28, 1920, a farm hand at Arlington, Va., noticed it on sweet potato and expressed the usual apprehension of injury.

July 21, 1922, Prof. H. F. Wilson, Madison, Wis., reported this species as being very common in Wisconsin and as creating considerable apprehension. Later he wrote that reports of damage were obviously erroneous and that the insect was not in reality a pest in that State.

NATURAL ENEMIES

The United States Bureau of Entomology has records of three natural enemies of this tortoise beetle; an egg parasite, a larval parasite, and a predacious bug.

Emersonella niveipes Girault.[4]—From egg masses collected by Miss Van Horn in the District of Columbia June 28, 1919, *Emersonella niveipes* Gir., a minute chalcidoid, began emerging July 12, 1919. From eggs collected by the writer September 5, 13 of these parasites emerged from one egg mass consisting of 19 eggs, in each case issuing from a round hole on one side near the top of the egg.

Masicera exilis Coquillett.—During July of 1907 several mature larvæ were observed in the District of Columbia on sweet potato leaves, from which the tachinid fly *Masicera exilis* issued July 19 to 29. July 2, 1919, the same species was reared from a larva from Arlington, Va.

Apateticus bracteatus Fitch.—July 26, 1909, Miss Julia D. Whiting, Deerfield, Mass., sent specimens of a large pentatomid bug, *Apateticus bracteatus* (fig. 4), which were observed attacking the larva of the Argus tortoise beetle in that vicinity. The nymphs transformed to adults August 4 to 7.

[4] Determined by A. B. Gahan.

The Biological Survey has found the Argus tortoise beetle in the stomachs of 14 species of birds, most often in those of the starling (*Sturnus vulgaris*) and kingbird (*Tyrannus tyrannus*).

GENERAL SUMMARY

The foliage of sweet potato, bindweed, and morning-glory is attacked by the adults and larvæ of the Argus tortoise beetle (*Chelymorpha cassidea*). Reports of attack to plants other than Convolvulaceae are in the main, if not entirely, erroneous.

The species has been studied in the District of Columbia. The eggs are deposited in clusters, varying from 16 to 28, on the lower surface of the leaves. They hatch in about 10 days into light yellow larvæ, which

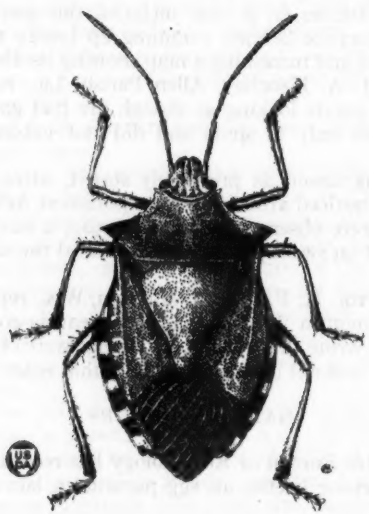


FIG. 4.—*Apateticus bracteatus*, a predacious bug enemy of the Argus tortoise beetle.

are gregarious and feed on the lower side of the foliage. In about three weeks they become mature and develop into similarly colored pupæ, which in from 7 to 9 days give forth the beetle. The species is evidently single-brooded in the North, double-brooded southward, and in the District of Columbia there is an exceptionally small second generation.

The Argus tortoise beetle is seldom so abundant as to be very destructive, plants readily recovering from its attack. The insect may be hand-picked in all stages and larvæ and adults can be killed with arsenicals.

LITERATURE CITED

(1) BARBER, H. S.
1916. A REVIEW OF NORTH AMERICAN TORTOISE BEETLES. *In* Proc. Ent. Soc. Wash., v. 18, no. 2, p. 113–127, 1 pl.

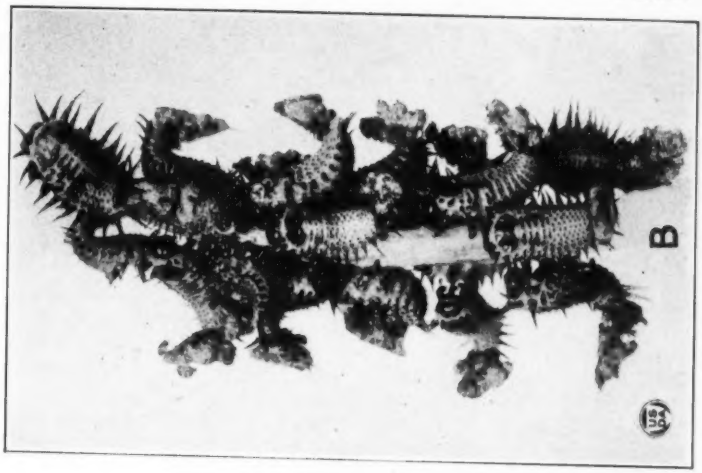
(2) CHITTENDEN, F. H.
1897. NOTES ON CERTAIN SPECIES OF COLEOPTERA THAT ATTACK USEFUL PLANTS. *In* U. S. Dept. Agr., Div. Ent. Bul. 9, n. s., p. 20–24.

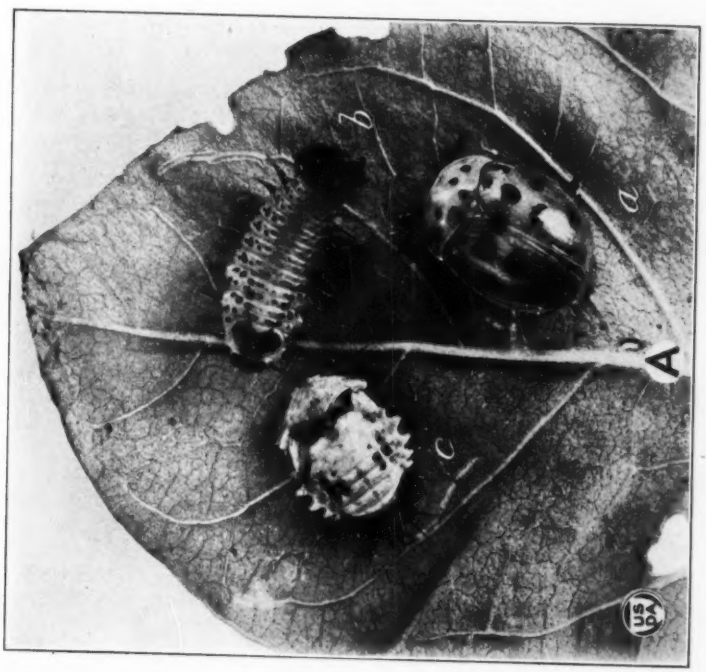
(3) FABRICIUS, Jo. Christ.
1775. SYSTEMA ENTOMOLOGIAE, SISTENS INSECTORUM CLASSES, ORDINES, GENERA, SPECIES, ADJECTIS SYNONYMIS, LOCIS, DESCRIPTIONIBUS, OBSERVATIONIBUS. 268 p. Flensburgi et Lipsiae.
(4) FALL, H. C., and COCKERELL, T. D. A.
1907. THE COLEOPTERA OF NEW MEXICO. *In* Trans. Amer. Ent. Soc., v. 33, p. 145–172.
(5) FORBES, S. A.
1905. TWENTY-THIRD REPORT OF THE STATE ENTOMOLOGIST ON THE NOXIOUS AND BENEFICIAL INSECTS OF THE STATE OF ILLINOIS. 273 p., illus.
(6) HARRINGTON, W. Hague.
1879. [NOTE ON CHELYMORPHA CRIBRARIA.] *In* Canad. Ent., v. 11, no. 6, p. 120.
(7) KNAB, Frederick.
1909. NUPTIAL COLORS IN THE CHRYSOMELIDAE. *In* Proc. Ent. Soc. Wash., v. 11, no. 3, p. 151–153.
(8) LINTNER, J. A.
1887. MILKWEED BEETLE WITH BAD HABITS. *In* Cultivator and Country Gent., v. 52, p. 673.
(9) LUGGER, Otto.
1899. BEETLES INJURIOUS TO FRUIT-PRODUCING PLANTS. Minn. Agr. Exp. Sta. Bul. 66, 332 p., illus.
(10) PACKARD, A. S.
1869. GUIDE TO THE STUDY OF INSECTS, AND A TREATISE ON THOSE INJURIOUS AND BENEFICIAL TO CROPS. 702 p., illus. Salem, Mass.
(11) RILEY, Charles V.
1870. SECOND ANNUAL REPORT ON THE NOXIOUS, BENEFICIAL, AND OTHER INSECTS, OF THE STATE OF MISSOURI. 135 p., illus.
(12) ———
1870. TORTOISE BEETLES. *In* Amer. Ent., v. 2, p. 2–5, 3 fig.
(13) SANDERSON, E. Dwight.
1899. SWEET POTATO INSECTS. Md. Agr. Exp. Sta. Bul. 59, p. 129–146, fig. 28–43.
(14) WEBSTER, F. M.
1893. INSECTS AFFECTING THE BLACKBERRY AND RASPBERRY. Ohio Agr. Exp. Sta. Bul. 45, p. 151–217, 38 fig.
(15) ——— and MALLY, C. W.
1898. INSECTS OF THE YEAR IN OHIO. *In* U. S. Dept. Agr., Div. Ent. Bul. 17, n. s., p. 98–102.

PLATE I

Chelymorpha cassidea

A.—Sweet-potato leaf showing (*a*) adult, (*b*) larva, and (*c*) pupa.
B.—Half-grown larvæ preparing to molt at tip of stem on which they have devoured the foliage.


B


A
a
b
c

SEED-COLOR INHERITANCE IN CERTAIN GRAIN-SORGHUM CROSSES[1]

By JOHN B. SIEGLINGER,

Assistant Agronomist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Color of seed is one of the most evident varietal characters of the grain sorghums. Though it is not of major economic importance, yet it does influence the popularity of a variety. On the Kansas City and Wichita markets yellow milo is generally quoted at 10 cents per 100 pounds higher than white kafir. On the other hand, brown-seeded sorghums do not command as high a price or sell as readily as do the varieties with white, yellow, or red seeds.

Naturally, color of seed has attracted the attention of the plant breeder working with sorghums as it has that of workers with most other cereal crops. Graham (*2*)[2] of India investigated the inheritance of seed color in natural and artificial sorghum crosses. The colors studied were red, yellow, and white. Red was found to be dominant to yellow and white, and yellow dominant to white. In a cross between a yellow- and a white-seeded sorghum, the heterozygote was red seeded, segregating in the F_2 generation in a ratio of 9 red : 3 yellow : 4 white. Karper and Conner (*6*) and Sieglinger (*8*) determined the amount of cross-pollination in milo by taking advantage of the fact that the seed of the F_1 of a cross between white and yellow milo is yellow. Vinall and Cron (*9*), in reporting the inheritance of certain sorghum characters, state that a close agreement to a 9:7 dihybrid ratio for brown and white seed was obtained in crosses between Blackhull kafir and feterita. This color inheritance was attributed to two factors—*B*, a factor for brown color, carried by the Blackhull kafir, and *S*, a spreader factor carried by the feterita.

Conner and Karper (*1*), in a paper published after the original draft of this paper was written, reported on the inheritance of seed-coat color in the hybrids Dwarf Yellow × Dwarf White milo, Blackhull × Red kafir, and Blackhull × Pink kafir. They found the F_1 in the milo cross to be indistinguishable in color from the yellow parent, the F_2 segregating in the ratio of 3 yellow to 1 white. In the Blackhull kafir × Red kafir cross, the seeds borne by the F_1 plants were pale red, intermediate in color between those of the parents. The F_2 segregated in the ratio of 1 white, 2 pale red, and 1 red. The phenotypic classification of the material was substantiated by growing the F_3, practically all of the whites and reds remaining constant and the pale reds segregating. In the cross Blackhull kafir × Pink kafir the F_1 was intermediate in seed color between the parents, the F_2 segregating in the ratio of 1 white, 2 pale pink, and 1 pink. No hybrid vigor was noted in any of these three crosses between sorghum varieties, the parents apparently being similar in most respects except seed color.

[1] Accepted for publication November 19, 1923.

[2] Reference is made by numbers (*italic*) to "Literature cited," p. 64.

EXPERIMENTAL MATERIAL AND METHODS

Several crosses between different varieties of grain sorghums were made by the writer at the Woodward Field Station, Woodward, Okla., in 1919 and 1920. Some of these have been grown through the F_3 generation, while the remainder have been carried through the F_2 generation. The crosses discussed in this paper are (1) feterita × Sunrise kafir and its reciprocal, (2) Sunrise kafir × Blackhull kaoliang, (3) feterita × Red kafir, (4) Sunrise kafir × Red kafir, (5) White kafir × Red kafir, and (6) White kafir × Sunrise kafir.

SEED COLOR OF PARENT VARIETIES

Feterita kernels are chalky or starchy white in external appearance, but portions of the seeds frequently are stained a dark red. This discoloration is most apparent on that part of the kernel covered by the glumes. The kernels of the selection of Blackhull kaoliang used in this study have the same sublenticular shape and peculiar chalky white color as those of feterita. It was selected from the progeny of bulk seed of Barchet kaoliang obtained in 1915 at the Amarillo Cereal Field Station. From the shape and color of the seeds, it is believed to have arisen through natural crossing between this kaoliang and feterita.

Sunrise kafir has the typical seed color of the white-seeded kafirs, creamy white with a more glossy surface than that of feterita and the Blackhull kaoliang hybrid. White kernels of kafir usually are marked with reddish black spots which occur most often at the point of attachment of the style. These spots vary in size and seem to be influenced to some extent by the environmental conditions that obtain during kernel development.

Red kafir kernels are dark brownish orange or brownish red with a smooth or glossy appearance. Frequently the color is not uniform over the kernel, due to bleaching where exposed to the weather.

Kernels of feterita and Blackhull kaoliang possess a layer of comparatively thick brown cells directly outside of the aleurone layer. This layer of cells is designated as the nucellar or hyalin layer, by Winton (*10*), the seed coat (Samenschale) by Harz (*5*, p. 1249–1254), and hyalin layer (Hyalinschicte) by Mitlacher (*7*), and probably is analogous to the inner integument in Johnson grass as described by Harrington and Crocker (*3*). Whatever this layer occurring directly outside of the aleurone layer may be called, it consists of relatively large cells containing a dark brown pigment. In Sunrise and other white kafirs, and in Red kafir, this layer of dark brown cells is absent. Winton (*10*) made a microscopic examination of the seeds of 11 varieties of sorghum. Of these, a nucellar layer was found in three varieties of broomcorn, Early Amber and Early Orange sorgos and Brown durra. The other varieties, White and Red kafir, White and Yellow milo, and White durra, had no nucellar layer. Winton's studies and the writer's examination of kernels of many sorghum varieties indicate that brown seed color is an indication of a brown nucellar layer. Feterita, Blackhull kaoliang, and Dwarf hegari have white seeds with a brown nucellar layer. A casual examination shows that these three white-seeded varieties which have a nucellar layer are chalky or starchy white in color, differing in this respect from many other white-seeded sorghums.

The writer is indebted to Dr. Carleton R. Ball for examining the seeds of several different introductions of white-seeded kaoliangs from China. He determines that two closely related varieties having black glumes and white seeds, namely, Barchet (C. I. 310, S. P. I. 22912) and Korean Blackhull (C. I. 412, S. P. I. 27553) both possess a brown nucellar layer like that of feterita. On the other hand, Brill Blackhull (C. I. 120, S. P. I. 17920), Mukden White (C. I. 190 and 272, S. P. I. 18610 and 21077), and an unclassified white-seeded variety (C. I. 350, S. P. I. 29166) show no trace of a brown nucellar layer. He reports also that the seeds of the varieties having the brown layer are whiter and more chalky in appearance than those not showing it, thus resembling feterita seeds, while the others more nearly resemble kafir seeds.

EXPERIMENTAL RESULTS

SUNRISE KAFIR × FETERITA AND THE RECIPROCAL CROSS

Reciprocal crosses were made between Sunrise kafir and feterita in 1919. A number of crossed seeds were obtained, all of which resembled the pistillate parent, there being no indication of xenia. Some of these seeds were sown in 1920, and several of the resulting F_1 heads were selfed. The seed produced on the F_1 plants was tan or light brown, with a brown nucellar layer similar to that of feterita. As neither of the parents had brown seeds, the production of brown seeds in the F_1 was not expected.

TABLE I.—*Distribution of seed-color in the F_2 generation of the cross Sunrise kafir × feterita, and its reciprocal*

Designation.	Year.	Plants. Total number.	Having seeds with— Brown nucellar layer present. Seeds brown.	Brown nucellar layer present. Seeds white.	Brown nucellar layer absent, seeds white.
Sunrise kafir × feterita	1921	179	111	29	39
Do	1921	145	85	26	34
Feterita × Sunrise kafir	1921	193	111	33	49
Sunrise kafir × feterita	1922	156	100	24	32
Feterita × Sunrise kafir	1922	172	93	31	48
Do	1922	174	101	34	39
Do	1922	163	97	34	32
Total		1,182	698	211	273
Calculated (9:3:4 ratio)			664.9	221.6	295.5
Deviation			33.1	10.6	22.5

Seeds from two of the F_1 heads of Sunrise kafir × feterita and one head of the reciprocal cross were sown in head rows in 1921. Preliminary counts of the F_2 heads in the plat showed clearly that the seed-color character was influenced by more than one factor. When mature, one head was harvested from each plant for a study of seed color. The F_2 heads of these crosses were readily placed in three groups according

to seed color—(1) brown or light brown, with a brown nucellar layer; (2) chalky or starchy white, with a brown nucellar layer, similar to the feterita parent; and (3) creamy white with no brown nucellar layer, similar to the Sunrise kafir parent. To determine the presence or absence of the brown nucellar layer, a kernel was taken from each head and the outer coat scraped off with a knife. This method of determination proved to be accurate and satisfactory.

Some of the crossed seeds obtained in 1919 were not sown until 1921, when several F_1 heads were selfed. One head row of F_2 plants of Sunrise kafir × feterita and three head rows of the reciprocal cross were grown in 1922. This F_2 material was similar to that obtained in the preceding year. Data on the distribution of seed color in the F_2 of this cross and its reciprocal are shown in Table I.

Using the method of Harris (*4*) for testing the goodness of fit of Mendelian ratios, $\chi^2 = 3.86$ and $p = .1476$, or a variation of the size obtained might be expected once in seven cases, due to random sampling. It appears that the segregation of color factors in a Sunrise kafir × feterita cross was according to the dihybrid ratio of 9: 3: 4. To explain this inheritance the following factors may be assumed:

B, a factor for brown nucellar layer which also may cause brown in the epidermis if *S* is present. Its allelomorph *b*, gives kernels without a brown nucellar layer.

S, a factor for smooth or glossy pericarp. When *S* is present the pericarp is glossy and may be creamy white, as in white kafir, or may carry other colors. Its allelomorph *s*, gives a chalky white pericarp. Brown does not appear in the pericarp of an *ss* plant.

The parents and F_1 hybrids, so far as shown by the results of this cross, then would have the following factorial composition:

Feterita ..... *BBss.*
Kafir ..... *bbSS.*
F_1 hybrid ..... *BbSs.*

The F_1 kernels having one *B* factor have a brown nucellar layer. As an *S* factor also is present, the brown color appears in the epidermis. The F_2 results seem to substantiate this hypothesis. The results expected in the F_2 generation, from an independent recombination of the two factors just designated, would produce three phenotypes classified as follows:

Brown seeded, with brown nucellar layer.	Chalky white seeded, with brown nucellar layer.	Glossy white seeded, with no brown nucellar layer.
1 BBSS	1 BBss	1 bbSS
2 BBSs	2 Bbss	2 bbSs
2 BbSS	—	1 bbss
4 BbSs	3	—
—		4
9		

The data presented in Table I are in substantial agreement with the hypothesis here presented. The *bbss* seeds with a chalky white color, but with no brown nucellar layer, because of weather effects, are difficult to distinguish when this chalky white is not over a brown nucellar layer,

and were classified with the glossy white phenotype with no nucellar layer.

A number of selfed heads were obtained from the F_2 rows of Sunrise kafir × feterita and its reciprocal grown in 1921. Seed from these and from several unbagged heads were sown in head rows in 1922 to determine the behavior of the material in the F_3 generation. Descriptions of the seed from the F_2 heads and the phenotypic distribution of the F_3 progenies grown from them in 1922 are shown in Table II.

TABLE II.—*Description of F_2 seed heads of the cross, Sunrise kafir × feterita, and its reciprocal, and distribution of the progenies obtained from these heads into seed-color classes in 1922*

Description of seed heads.	Distribution of F_3 progeny.			Factorial composition.
	Brown nucellar layer present.		Brown nucellar layer absent, white seeds.	
	Brown seeds.	Chalky white seeds.		
Sunrise kafir × feterita (row 1 in 1921):				
1. Not bagged, white, no nucellar layer...	0	0	40	bbss, bbSs, or bbSS
2. Not bagged, brown, nucellar layer.....	86	27	0	BBsS
3. Selfed, brown, brown nucellar layer...	57	25	22	BbSs
4. Do.....	124	0	0	BBSS
5. Not bagged, white, brown nucellar layer	0	78	23	Bbss
Sunrise kafir × feterita (row 2 in 1921):				
1. Selfed, brown, brown nucellar layer...	74	0	27	BbSS
2. Do.....	113	43	48	BbSs
3. Do.....	82	30	40	BbSs
4. Not bagged, white, no nucellar layer...	0	0	123	bbss, bbSs, or bbSS
5. Selfed, brown, brown nucellar layer....	60	23	34	BbSs
6. Do.....	114	0	33	BbSS
7. Selfed, white, brown nucellar layer....	0	148	0	BBss
Feterita × Sunrise kafir:				
1. Selfed, white, brown nucellar layer....	0	90	26	Bbss
2. Do.....	0	79	29	Bbss
3. Do.....	0	89	0	BBss
4. Selfed, white, no nucellar layer.....	0	0	133	bbss, bbSs, or bbSS
5. Selfed, white, brown nucellar layer....	0	94	0	BBss
6. Selfed, brown, brown nucellar layer....	85	31	0	BBSs

The factorial constitution of most of the F_2 heads used as seed can be determined definitely from the F_3 results. This is given in the last column of Table II, on the basis of the two-factor hypothesis previously outlined, and will account for all observed types. These two factors, therefore, not only account for the surface color of the kernel, but also for the presence or absence of the very apparent brown nucellar layer.

The ratios obtained in the F_3 generation, while not exact, are as close to the expected as such small numbers of plants would require. Three possible factor combinations are suggested for the parent heads of the

three pure white F_3 progenies. Any of the factor combinations indicated for these F_2 heads would produce all white-seeded plants, without brown nucellar layer, in the F_3 generation, and consequently the specific one can not be determined.

SUNRISE KAFIR × BLACKHULL KAOLIANG

A cross was made in 1919 between Sunrise kafir and Blackhull kaoliang, using Sunrise kafir as the pistillate parent. Reciprocal crosses were attempted, but the Blackhull kaoliang spikelets seemed to be very susceptible to mutilation and the emasculated flowers dried without producing seed. Part of the crossed seeds were sown in 1920 and the remainder in 1921.

The F_1 kernels of the Sunrise kafir × Blackhull kaoliang cross were of a tan or brown surface color. A brown nucellar layer also was present. The similarity in appearance of feterita and Blackhull kaoliang kernels and the similarity in the color of the seeds of F_1 crosses between these varieties and Sunrise kafir indicate that the same or very similar color factors are present in feterita and Blackhull kaoliang. As previously stated, the strain of kaoliang used probably is a selection from a natural hybrid between Barchet kaoliang and feterita.

Seed from two selfed heads of the F_1 generation grown in 1920 were sown in head rows in 1921, and two similar head rows were grown in 1922 from F_1 plants selfed in 1921. The data on seed color obtained from the F_2 progenies in 1921 and 1922 are presented in Table III.

TABLE III.—*Distribution of seed color in the F_2 generation of the cross Sunrise kafir × Blackhull kaoliang, grown in 1921 and 1922*

Row number.	Year.	Plants.			
		Total number.	With seeds having—		
			Brown nucellar layer present.		Brown nucellar layer absent, seeds white.
			Seeds brown.	Seeds white.	
1	1921	224	132	44	48
2	1921	226	132	47	47
1	1922	227	133	46	48
2	1922	250	159	43	48
Total		927	556	180	191
Calculated (9:3:4 ratio)			521	174	232
Deviation			35	6	41

X^2=9.803. P=.0076, 1 in 131, or 0.76 per cent.

The results indicate that the segregation of seed-color factors in the Sunrise kafir × Blackhull kaoliang cross is similar to that in the crosses between Sunrise kafir and feterita. While the data in Table III diverge somewhat widely from the calculated ratios, yet in view of similar data from other crosses the agreement is close enough to confirm the two-factor hypothesis.

Seed of four selfed F_2 heads was sown in head rows in 1922 and the data shown in Table IV were secured.

TABLE IV.—*Description of F_2 seed heads of the cross Sunrise kafir × Blackhull kaoliang and distribution of the F_3 progenies of these heads into seed-color classes in 1922*

Description of F_2 seed heads.	Plants with seeds having—			Factorial composition.
	Brown nucellar layer present.		Brown nucellar layer absent, seeds white.	
	Seeds brown.	Seeds white.		
Selfed, brown, brown nucellar layer	124	43	51	BbSs
Do	103	35	46	BbSs
Selfed, white, brown nucellar layer	0	64	11	Bbss
Do	0	148	47	Bbss

The distribution of progeny from all four rows of F_2 material in 1921 and 1922 differed considerably from the calculated 9:3:4 ratio, there being a surplus of brown-seeded plants with brown nucellar layer and a deficiency of white-seeded plants without brown nucellar layer. However, rows 1 and 2 of the F_3 generation closely approach a perfect 9:3:4 distribution, the actual distribution of 402 plants being 227:78:97, as compared with a calculated distribution of 226:75:101.

From the data reported here on F_1, F_2, and F_3 material of crosses between Sunrise kafir and feterita and between Sunrise kafir and Blackhull kaoliang, it appears that the seed color of feterita and Blackhull kaoliang is due to the presence of two color factors designated as *BB* and *ss*, while the seed color of Sunrise kafir is due to two color factors designated as *bb* and *SS*.

FETERITA × RED KAFIR

In view of the unexpected occurrence of brown color in crosses having feterita or Blackhull kaoliang as a parent, some data on a cross between feterita and Red kafir are presented to further substantiate the hypothesis that feterita carries a factor which produces a brown color.

A cross was made in 1920 between feterita and Red kafir, feterita being the pistillate parent. The kernels in the F_1 heads produced in 1921 ere brown, though the intensity of the brown varied, depending on the extent of weathering. On that part of the seed covered by the glumes there was quite a tinge of red color.

Seed from four selfed F_1 heads was sown in head rows in 1922. One head from each F_2 plant was harvested and taken to the laboratory, where color of seed was recorded. Many variations and tints of color were present in the F_2 material and it was necessary to make rather broad classes to obtain accuracy. The classes used were as follows:

Brown seeds, with brown nucellar layer.
White seeds, with brown nucellar layer.
Red and pink seeds, with no brown nucellar layer.
White seeds, with no brown nucellar layer.

All the F_2 heads could be placed in one of the above color classes with a fair degree of accuracy. The results of this classification of the F_2 progenies of the feterita × Red kafir cross are shown in Table V.

TABLE V.—*Distribution of seed color in the F_2 generation of the cross feterita × Red kafir, grown in 1922*

Row number.	Plants.				
	Total number.	Having seeds with—			
		Brown nucellar layer present.		Brown nucellar layer absent.	
		Seeds, brown.	Seeds, white.	Seeds, red or pink.	Seeds, white.
1	165	114	7	33	11
2	138	103	7	20	8
3	174	126	9	26	13
4	121	87	6	22	6
Total	598	430	29	101	38
Calculated (45:3:12:4 ratio)		421	28	112	37

X^2=1.335. P =0.72468.

A study of the color distribution in the F_2 generation of the cross between feterita and Red kafir indicates that the two color factors involved in the cross between feterita and White kafir are concerned, and that in addition a third color factor for red, introduced by the Red kafir parent, must also be considered. The color factors in feterita which are concerned in this cross may be designated as *BBssrr*, while those of Red kafir may be designated as *bbSSRR*, *R* being the factor for red color, as present in Red kafir, and *r* its recessive allelomorph for absence of the red color. The F_1 cross then would be *BbSsRr*. The probable effect on seed color of the *B*, *b*, and *S*, *s* factors was stated in the discussion of the previous crosses and the *R*, *r*, factors will be considered more fully later. A study of the feterita × Red kafir cross indicates that when the factor for brown nucellar layer is present with the factor for red, *R*, the color of the kernel is brown rather than red, and the phenotype then is brown.

On the basis of three color factors in 64 individuals of the F_2 generation there should be 45 plants with brown seeds with brown nucellar layer, 3 plants with white seeds with brown nucellar layer, 12 plants with red or light red seeds and no brown nucellar layer, and 4 plants with white seeds without brown nucellar layer. It must be assumed that the presence of either the *S* or the *R* factor will cause the brown color to appear in the epidermis of the seed. The actual color distribution obtained in the four F_2 rows, as compared with the theoretical results, is shown in Table V. A deviation of this magnitude might be expected to occur once in 1.38 cases as the result of random sampling.

The fit of the observed to calculated distribution of the color phenotypes is very close, in fact much better than usually would be expected, considering the relatively small number of individuals, 598. The con-

clusion seems justified that in the feterita × Red kafir cross there are three factors influencing the color of seed, and that the three color factors are independently inherited, i. e., there is no evidence of linkage.

The possible combinations of three factor pairs, *Bb*, *Ss*, *Rr*, in the F_2 generation are grouped under four phenotypes, as follows:

Brown surface, brown nucellar layer present.	White surface, brown nucellar layer present.	Red or pink surface, brown nucellar layer absent.	White surface, brown nucellar layer absent.
1 BBSSRR	1 BBssrr	1 bbSSRR	1 bbSSrr
2 BbSSRR	2 Bbssrr	2 bbSSRr	2 bbSsrr
2 BBSSRr	3	2 bbSsRR	1 bbssrr
4 BbSSRr		4 bbSsRr	4
2 BBSsRR		1 bbssRR	
4 BbSsRR		2 bbssRr	
1 BBSSrr		12	
2 BbSSrr			
4 BBSsRr			
8 BbSsRr			
2 BBSsrr			
4 BbSsrr			
1 BBssRR[a]			
2 BbssRR[a]			
2 BBssRr[a]			
4 BbssRr[a]			
45			

[a] Under the climatic conditions occurring in 1922, there was no evidence of a phenotype similar to feterita but carrying red in the pericarp. It was concluded, therefore, that an *R* factor with a *B* factor in the presence of *ss* gave a seed which was classified with the phenotype having brown surface color and brown nucellar layer. However, in 1923, the F_3 material from the same source as in 1922 showed a number of plants whose seeds possessed a brown nucellar layer and had a white outer color marked with red. These doubtless are individuals having the factorial constitution of BBssRR, BbssRR, BBssRr, or BbssRr.

SUNRISE KAFIR × RED KAFIR

In the discussion of the crosses previously described, in which feterita or Blackhull kaoliang was one parent and Sunrise or Red kafir the other parent, it was shown that brown seeds were obtained in the F_1 generation and in more than half of the variates in the F_2 generation.

The cross Sunrise kafir × Red kafir was made in 1920. A few crossed seeds were sown in 1921, from which two selfed F_1 heads were obtained. The seed from these was sown in head rows in 1922, and the segregation for seed color in the F_2 generation of this cross is shown in Table VI.

TABLE VI.—*Distribution of seed-color in the F_2 generation of the cross Sunrise kafir × Red kafir, grown in 1922*

Row number.	Total number of plants.	Plants with red and tinted seed.	Plants with white seed.
1	144	111	33
2	143	101	42
Total	287	212	75
Expected (3:1 ratio)		215	72

Deviation, 3 ± 4.95. $\frac{\text{Dev.}}{\text{P. E.}} = 0.606$.

The results shown in Table VI indicate that Sunrise kafir and Red kafir differ by a single main factor for seed color. The factors influencing color in the Sunrise × Red kafir cross may be designated as *rr* for Sunrise kafir and *RR* for Red kafir. The seed color of the cross is due to the combination *Rr* in the F_1 and the segregation of the *Rr* factor pair in the F_2 generation in the monohybrid ratio of 3:1. While it seldom is advisable to formulate a factorial hypothesis on the behavior of only 287 F_2 individuals, in this instance it seems safe to do so, as the data on other crosses, White kafir × Red kafir and White kafir × Sunrise kafir, reported in this paper, bear out this theory. Sunrise kafir and White kafir have a similar seed color and the color results obtained in White kafir crosses should be the same as those in which Sunrise kafir is used.

WHITE KAFIR × RED KAFIR

A cross was made in 1919 between White kafir and Red kafir, the White kafir being the pistillate parent. Two F_2 rows were produced in 1921 from seed of selfed F_1 heads. The segregation of seed-color in the plants in these two head rows is shown in Table VII.

TABLE VII.—*Distribution of seed color in the F_2 generation of the cross White kafir × Red kafir, grown in 1921*

Row number.	Total number of plants.	Plants with red seeds.	Plants with white seeds.
1	202	150	52
2	198	151	47
Total	400	301	99
Expected (3:1 ratio)		300	100

Deviation, 1 ± 5.84. $\frac{\text{Dev.}}{\text{P. E.}}$, $= 0.171$.

Six selfed heads from each of the two F_2 rows of this cross were used for sowing head rows in 1922. These 12 heads were selected for other characters than color of seed, the selection not being random, but seed color was recorded both for the parent heads and the F_3 progenies. The data obtained in the F_3 generation are shown in Table VIII.

Six of the F_2 heads were classed as red and produced nothing but red-seeded progenies, with a total of 659 individuals. The three white-seeded F_2 heads produced a total of 364 white-seeded individuals. The three parent heads classified as light red obviously were the heterozygotes, producing red- and white-seeded plants in a ratio fairly close to 3:1. As noted, the parent heads were not random selections, so that the fact that the progenies did not occur in a 1:2:1 ratio of dominants, heterozygotes, and recessives is immaterial. Especially is this true in view of the fact that the heterozygotes can be determined by their intermediacy.

TABLE VIII.—*Distribution of seed-color in the F_2 generation of the cross White kafir × Red kafir in 1922*

Designation.	Total number of plants.	Number of plants with—	
		Red seeds.	White seeds.
F_2 heads, red seeds:			
1–2	120	120	0
1–5	117	117	0
2–1	97	97	0
2–2	107	107	0
2–3	104	104	0
2–5	114	114	0
Total, 6 progenies	659	659	0
F_2 heads, white seeds:			
1–3	135	0	135
1–6	114	0	114
2–4	115	0	115
Total, 3 progenies	364	0	364
F_2 heads, light red seeds:			
1–1	117	85	32
1–4	110	81	29
2–6	110	81	29
Total, 3 progenies	337	247	90
Expected (3:1 ratio)	.....	253	84

Deviation, 6 ± 5.36. $\frac{\text{Dev.}}{\text{P. E.}} = 1.119$.

WHITE KAFIR × SUNRISE KAFIR

A cross was made between White kafir and Sunrise kafir in 1919. The F_1, F_2, and F_3 plants grown from this cross produced kernels typical of white kafir in color, indicating that the genetic factors governing color of seed in these two varieties were the same.

SUMMARY

The seeds of feterita and Blackhull kaoliang are a chalky opaque white. These varieties also have a well developed brown nucellar layer lying directly outside of the aleurone layer.

Seeds of Sunrise kafir are creamy white, with a smooth or glossy pericarp. No brown nucellar layer is present in the kernels of this variety.

Crosses between feterita or Blackhull kaoliang and Sunrise kafir produce F_1 plants with light brown seeds with brown nucellar layer.

In the F_2 generation of these crosses segregation occurs, the seed color of the three phenotypes being (*a*) brown seeds with brown nucellar layer, (*b*) white seeds with brown nucellar layer, and (*c*) white seeds with no brown nucellar layer. The ratio of these classes is approximately 9:3:4.

Results obtained in the F_3 generation confirm the two-factor hypothesis of color inheritance in these crosses.

Red kafir seed is of a dark red color, with no brown nucellar layer. A cross between feterita and Red kafir produces a reddish-brown kernel with brown nucellar layer in the F_1 generation.

In the F_2 generation of the feterita × Red kafir cross, four phenotypes are obtained, these being (*a*) brown with brown nucellar layer, (*b*) white with brown nucellus, (*c*) red and pink with no brown nucellus, and (*d*) white with no brown nucellus. The approximate ratio of these phenotypes is 45:3:12:4, indicating that three main factors influence the inheritance of seed color in this cross.

In the crosses Sunrise kafir × Red kafir and White kafir × Red kafir, segregation in the F_2 generation occurs in a simple monohybrid ratio of three red-seeded plants to one white-seeded plant.

Crosses between White kafir and Sunrise kafir show that the seed-color of these two varieties is genetically identical.

The factors concerned in the inheritance of seed color in these sorghum crosses are *B*, a factor for brown nucellar layer, which produces brown color in the epidermis in the presence of *S*; *b*, its allelomorph, giving no brown nucellar layer when homozygous; *S*, a factor which will cause the development of color in the epidermis of the seed; *s*, its allelomorph, which, when present in the homozygous condition, will prevent the development of the brown color in the epidermis, *R*, a factor for dark red, which, when present, causes the development of red color in the epidermis; and *r*, its allelomorph, absence of red color.

The factorial constitution of feterita and Blackhull kaoliang with respect to seed color is *BBssrr*, of Sunrise and White kafir, *bbSSrr*, and of Red kafir, *bbSSRR*.

LITERATURE CITED

(1) Conner, A. B., and Karper, R. E.
1923. The inheritance of seed coat color in certain crosses in grain sorghum. *In* Jour. Amer. Soc. Agron., v. 15, p. 338–344. Literature cited, p. 344.

(2) Graham, R. J. Douglas.
1916. Pollination and cross-fertilization in the juar plant, (Andropogon sorghum, brot.). *In* Mem. Dept. Agr. India Bot. Ser., v. 8, p. 201–216, 1 pl.

(3) Harrington, George T., and Crocker, William.
1923. Structure, physical characteristics, and composition of the pericarp and integument of Johnson grass seed in relation to its physiology. *In* Jour. Agr. Research, v. 23, p. 193–222, 10 fig. Literature cited, p. 221–222.

(4) Harris, J. Arthur.
1912. A simple test of the goodness of fit of Mendelian ratios. *In* Amer. Nat., v. 46, p. 741–745.

(5) Harz, C. O.
1885. Landwirthshaftliche samenkunde. ix, 1362 p., 201 fig. Berlin.

(6) Karper, R. E., and Conner, A. B.
1919. Natural cross-pollination in milo. *In* Jour. Amer. Soc. Agron., v. 11, p. 257–259.

(7) Mitlacher, Wilhelm.
1901. Ueber einige exotische gramineenfrüchte, die zur menschlichen nahrung dienen. II. Andropogoneae. *In* Ztschr. Allg. Oesterr. Apoth. Ver., Jahrg. 39, p. 831–836.

(8) Sieglinger, John B.
1921. Cross-pollination of milo in adjoining rows. *In* Jour. Amer. Soc. Agron., v. 13, p. 280–282.

(9) Vinall, H. N., and Cron, A. B.
1921. Improvement of sorghums by hybridization. *In* Jour. Heredity, v. 12, p. 435–443, 6 fig.

(10) Winton, A. L.
1903. The anatomy of the fruit of certain cultivated sorghums. *In* Conn. Agr. Exp. Sta. 26th Ann. Rpt., 1902, p. 326–338, fig. 37–44.

THE EUROPEAN CORN BORER, PYRAUSTA NUBILALIS HBN., VERSUS THE CORN EARWORM, HELIOTHIS OBSOLETA FAB.[1]

By GEO. W. BARBER

Scientific Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture

The spread of the European corn borer, *Pyrausta nubilalis* Hbn., in the northeastern portion of the United States, during the last few years, creates interest in what will happen when this insect reaches the sections of the country where the corn earworm, *Heliothis obsoleta* Fab., seriously damages the corn crop. In the area infested by the European corn borer at present the corn earworm is present annually, but usually in small numbers, and seldom does serious injury to the corn.

In the late summer and fall of 1921 the corn earworm was abundant in late sweetcorn and in field corn in eastern Massachusetts. This was probably due to the occurrence of a second brood of the insect, which apparently was made possible either by the rather mild preceding winter or by the abnormally long growing season of that year. The infestation in some fields ran as high as 90 per cent of the ears, and injury was very apparent over a considerable area.

In the same season the European corn borer was also destructive in certain fields infested by the corn earworm, forecasting the condition which one might expect to find in the southern portion of the Corn Belt should the European corn borer ever reach that section.

Larvæ of both species were commonly found feeding in and on the same ears of corn, frequently their burrows uniting and adjoining, while the larvæ were in close proximity, each apparently too much occupied with the feast to be mindful of its neighbor.

Although not present in such large numbers, the larvæ of the corn earworm were able by the time they completed feeding to inflict more actual grain loss than the larvæ of the European corn borer, because of their greater feeding capacity and because their work was, for the most part, concentrated on the kernels, whereas the European corn borer larvæ feed throughout all parts of the ear and stalk, thus having a much wider range of activity. The feeding of the European corn borer larvæ, however, results frequently in more injury than is apparent in actual destruction of the grain, by injuring the stalk and particularly the peduncle of the ear. This often results in a reduction of the number of kernels forming on the ear. In 1921 many of the ears showed undeveloped kernels, particularly at the tips, which was undoubtedly due, at least in part, to the injury to other parts of the plants by the European corn borer.

A field of about an acre of Longfellow flint corn was used to obtain the information contained in this paper. The ears studied were selected at random and showed the average condition of the ears of the whole field. Ears shown in Plate 1 are average ears selected to show different types of injury rather than a maximum amount of damage.

When the counts shown in Table I were made (October 14, 1921) the corn earworm larvæ, in nearly all cases, were feeding in the ears. By October 20, 1921, when Table II was prepared, many of the larvæ of

[1] Accepted for publication Nov. 19, 1923.

the corn earworm had deserted the ears to pupate. The injury, it will be observed, had increased considerably by the second date. On October 31, 1921, practically all of the larvæ of this species had left the ears, and those that remained were nearly all dead as a result of heavy frosts. The larvæ of the European corn borer, on the other hand, had continued feeding during this time, so that the damage that might be credited to this species was, by the last date, probably as great or greater than that done by the corn earworm. An actual count, like that of Table II, was not practicable, however, since it was not then possible to recognize the injury occasioned by each species.

Tables I and II show in what parts of the ears the larvæ of the two species were found and the damage that they had occasioned. For this purpose the ear has been divided into husk, peduncle, silk, cob, and grain. For the purpose of recording more accurately the exact region of injury to the grain with relation to the ear, the latter has been further divided into tip, middle, and butt; the "tip" being the upper third, the "middle" the middle third, and the "butt" the lower third of the ear.

TABLE I.—*Infestation of field corn by the corn earworm, Heliothis obsoleta Fab., and the European corn borer, Pyrausta nubilalis Hbn., at Arlington, Mass., October 14, 1921*

[100 ears of Longfellow flint field corn]

Ear No.	Number and location of larvæ found.											Part of ear infested or injured.					Total grains on ear.	Grains destroyed.
	Corn earworm.					European corn borer.												
	Husk.	Silk.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.	Grain.		
1			1			1	1						+	+	+	o	+	336	20
2			3			1							+	+	+	o	o	272	0
3			2			1						1	+	+	+	o	+	264	29
4			2										o	+	+	o	+	256	0
5			2	2		1			1				+	+	+	+	+	320	39
6			1				1		1	1			o	+	+	+	+	240	3
7		1	1										o	o	+	o	+	320	14
8			2			2							+	+	+	o	+	256	30
9				2									o	o	+	o	+	264	19
10			1			1			1				+	+	+	+	+	416	35
11			2						1				o	+	+	+	o	296	0
12			2			3							+	+	+	o	+	240	28
13			2	1		3				1	3		+	+	+	o	+	288	66
14			1							1			o	+	+	o	+	384	12
15										1			o	+	+	o	o	240	0
16									1		2		o	+	+	+	+	256	30
17			5			1			1				+	+	+	+	+	284	37
18				2		1							+	+	+	o	+	208	47
19			2			1				1			+	+	+	o	+	272	35
20		2				1	1						+	+	+	o	o	336	0
21						1			1	1			+	+	o	+	+	224	22
22			3				1				2		o	+	+	o	+	224	48
23			2							1			o	+	+	o	+	344	18
24			2			1					4		+	+	+	o	+	264	36
25			1										o	+	+	o	+	272	15
26			2			1							+	+	+	o	+	344	10
27				1					1				o	+	+	+	+	248	16
28			3			4			1		1		+	+	+	+	+	240	50
29		1	1	2									o	+	+	o	+	224	33
30		3		2	1	1							+	+	+	o	+	224	33
31	1		2			1			1				+	+	+	+	+	192	24
32						1			1				+	+	+	+	+	208	3
33			2										o	o	+	o	+	312	16
34						1	1		1				+	+	+	+	o	320	0
35			1			2							+	+	+	o	+	284	18
36	1					1	3		1	3	2		+	+	+	+	+	344	32
37			1			1	1				1		+	+	+	o	+	208	34
38			1	1									o	+	+	o	+	368	20
39			2		1		2				1		o	+	+	o	+	280	67
40			2			2			1		1	1	+	+	+	+	+	216	24
41										1		2	o	+	+	o	+	352	15
42			1							1			o	+	+	o	+	232	24
43						1			1	1			+	+	+	+	+	284	5

TABLE I.—*Infestation of field corn by the corn earworm, Heliothis obsoleta Fab., and the European corn borer, Pyrausta nubilalis Hbn., at Arlington, Mass., October 14, 1921*—Continued

Ear No.	Number and location of larvæ found.												Part of ear infested or injured.					Total grains on ear.	Grains destroyed.
	Corn earworm.					European corn borer.													
	Husk.	Silk.	Tip.	Middle.	Butt.	Peduncle.	Husk	Silk.	Cob.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.	Grain.		
44	....	....	2	....	....	1	1	....	1	....	2	....	+	+	+	+	+	284	16
45	....	....	....	....	....	1	1	....	1	1	1	....	+	+	+	+	+	256	22
46	....	1	1	....	....	1	....	....	....	....	....	....	+	+	+	o	+	192	51
47	....	....	....	....	....	....	....	....	1	....	1	....	o	+	o	+	+	336	23
48	....	....	....	....	....	....	....	....	1	1	1	....	o	+	+	+	+	320	25
49	....	....	1	....	....	1	1	1	1	3	....	....	+	+	+	+	+	288	16
50	....	2	....	....	....	1	1	....	2	....	1	....	+	+	+	+	+	320	15
51	....	....	....	....	....	1	....	1	1	....	....	....	+	+	+	+	o	332	o
52	....	1	1	....	....	1	1	....	1	....	1	....	+	+	+	+	+	284	74
53	....	....	....	....	....	1	....	1	2	1	....	....	+	+	+	+	+	280	20
54	....	....	2	....	....	1	....	....	1	2	....	....	+	+	+	+	+	280	36
55	....	1	1	....	....	3	....	....	1	1	....	....	+	+	+	+	+	240	39
56	1	1	1	....	....	1	....	....	....	....	....	....	+	+	+	o	+	368	49
57	....	1	1	....	....	....	....	....	....	....	....	....	o	o	+	o	+	336	16
58	....	....	....	....	....	....	1	....	1	1	....	....	o	+	+	+	+	256	94
59	....	....	2	....	....	....	....	....	1	....	1	....	o	+	+	+	+	280	18
60	....	1	2	....	....	....	....	....	1	1	....	....	o	+	+	+	+	360	13
61	....	....	2	....	....	....	....	....	....	....	....	....	o	o	+	o	+	376	30
62	....	....	2	....	....	....	....	....	....	....	1	....	o	+	+	o	+	288	56
63	....	2	....	....	....	....	....	....	....	....	....	....	o	o	+	o	o	320	o
64	....	....	1	....	....	2	....	....	2	1	....	1	+	+	+	+	+	256	40
65	....	....	2	....	....	1	....	....	1	....	....	....	+	+	+	+	+	320	18
66	....	....	2	....	....	....	....	....	....	....	....	....	o	+	+	o	+	392	52
67	....	....	3	....	....	1	....	....	....	....	....	....	+	o	+	o	+	320	30
68	....	....	1	....	....	1	....	1	....	....	....	....	+	+	+	o	+	256	8
69	....	....	1	....	....	....	....	....	1	....	....	....	o	+	+	+	+	304	11
70	....	....	1	....	....	1	....	....	....	....	1	....	+	+	+	o	+	240	23
71	....	....	....	1	....	....	....	....	1	....	....	1	o	+	+	+	+	336	54
72	....	1	1	....	....	1	....	....	1	....	....	....	+	+	+	+	+	384	35
73	....	....	1	....	....	1	1	....	1	1	1	....	+	+	+	+	+	284	54
74	....	....	3	....	....	1	....	....	1	....	1	....	+	+	+	+	+	336	92
75	....	1	1	....	....	2	....	....	1	....	....	....	+	+	+	+	+	256	2
76	....	....	....	....	....	1	....	....	1	1	2	....	+	+	+	+	+	328	44
77	....	....	1	....	....	2	1	1	....	....	....	....	+	+	+	o	+	240	37
78	....	2	1	....	....	....	....	1	....	....	....	....	o	+	+	o	+	208	26
79	....	....	1	....	....	....	1	....	2	1	3	....	o	+	+	+	+	120	62
80	....	....	2	....	....	1	1	....	....	1	....	....	+	+	+	o	+	256	43
81	....	3	1	....	....	1	1	....	1	....	1	....	+	+	+	+	+	256	33
82	....	....	2	....	....	2	1	....	....	....	1	....	+	+	+	o	+	272	35
83	....	....	1	....	....	2	1	....	2	....	2	....	+	+	+	+	+	288	76
84	....	1	1	....	....	1	....	....	1	....	....	....	+	+	+	+	+	208	48
85	....	1	2	....	....	1	1	....	1	....	....	....	+	+	+	+	+	344	13
86	....	....	2	....	....	....	....	....	....	1	....	....	o	+	+	o	+	320	o
87	....	....	1	1	....	1	....	....	1	....	....	....	+	+	+	+	+	262	52
88	....	....	....	....	....	....	....	....	....	1	1	....	o	+	+	o	+	172	13
89	....	1	2	....	....	....	....	....	....	1	....	....	o	+	+	o	+	172	47
90	....	....	1	....	....	....	....	....	1	....	....	....	o	+	+	+	o	280	o
91	....	....	....	....	....	....	....	....	....	1	....	....	o	+	+	o	+	256	12
92	....	....	....	....	....	....	....	4	1	2	....	....	o	+	+	+	+	272	10
93	....	....	1	1	....	1	....	....	2	....	1	1	+	+	+	+	+	224	47
94	....	....	2	....	....	....	....	....	....	....	....	....	o	+	+	o	+	286	32
95	....	....	1	....	....	....	....	....	1	....	2	....	o	+	+	+	+	272	31
96	....	....	3	1	....	....	....	....	1	....	1	....	o	+	+	+	+	320	102
97	....	....	2	....	....	1	1	....	....	....	1	....	+	+	+	o	+	288	44
98	....	1	1	1	....	....	....	....	1	....	2	....	o	+	+	+	+	280	38
99	....	1	1	....	....	....	1	....	....	....	1	....	o	+	+	o	+	286	54
100	....	....	1	....	....	1	....	....	....	....	1	....	+	+	+	o	+	272	48

Per cent of ears infested:		
By either species	per cent..	100
By both species	do....	72
By European corn borer	do....	88
By corn earworm	do....	83
Average number of corn earworms per infested ear		2.07
Average number of European corn borers per infested ear		2.92
Total grain destroyed by both species	per cent..	10.5
Per cent of stalks infested (25 stalks)	do....	100
Infestation per stalk:		
Greatest	larvæ..	21
Least	do....	4
Average	do....	9.32
Greatest grain injury on any one ear	per cent..	31.9

TABLE II.—*Infestation of field corn by the corn earworm, Heliothis obsoleta Fab., and the European corn borer, Pyrausta nubilalis Hbn., at Arlington, Mass., October 20, 1921*

[50 ears of Longfellow flint field corn]

Ear No.	Number and location of larvæ found.												Part of ear infested or injured.					Total grains on ear.	Grains destroyed.	Grain destruction charged to the corn earworm.	Grain destruction charged to the European corn borer.
	Corn earworm.					European corn borer.															
	Husk.	Silk.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.	Grain.				
1	....	....	1	2	....	1	....	1	1	....	3	....	+	+	+	+	+	280	121	79	42
2	....	....	2	....	....	2	1	....	1	1	3	....	+	+	+	+	+	184	69	37	32
3	....	....	1	....	....	1	....	....	1	....	1	....	+	+	+	+	+	272	37	16	21
4	....	....	1	1	....	....	1	....	....	....	....	....	0	+	+	0	+	344	110	110	0
5	....	....	1	....	....	....	1	....	....	1	1	1	0	+	+	0	+	264	53	28	25
6	....	....	2	....	....	....	....	....	1	....	1	1	+	+	+	+	+	192	32	26	6
7	....	....	1	....	....	1	....	....	....	....	....	....	+	+	+	0	+	312	41	41	0
8	....	....	1	....	....	2	....	1	1	1	2	....	+	+	+	+	+	256	62	24	38
9	....	....	2	....	....	....	1	....	1	....	....	....	0	+	+	+	+	280	55	55	0
10	....	....	....	....	....	....	....	....	....	1	1	....	0	+	+	0	+	176	36	0	36
11	....	....	1	....	....	1	....	....	....	....	1	....	+	+	+	0	+	240	28	14	14
12	....	....	1	3	....	1	....	....	....	1	1	....	+	+	+	0	+	288	113	93	20
13	....	....	2	....	....	1	2	....	1	....	2	....	+	+	+	+	+	216	40	10	30
14	....	....	....	....	....	1	....	....	1	1	1	....	+	+	+	+	+	288	36	0	36
15	....	....	....	....	1	1	....	....	....	....	....	....	+	+	+	0	+	304	37	37	0
16	....	....	2	....	....	1	1	....	....	....	....	....	+	+	+	0	+	240	56	56	0
17	....	....	2	....	....	1	....	....	....	....	....	....	+	+	+	0	+	376	32	32	0
18	....	....	1	1	....	1	....	....	....	....	....	....	+	+	+	0	+	200	20	20	0
19	....	....	1	....	....	1	....	....	1	1	1	1	+	+	+	+	+	232	53	26	27
20	....	....	1	....	....	....	....	....	....	....	....	....	0	+	+	0	+	256	23	23	0
21	....	....	1	....	....	1	....	....	1	....	....	1	+	+	+	+	+	256	36	26	10
22	....	....	1	....	....	....	....	....	2	....	....	1	0	+	+	+	+	316	40	26	14
23	....	....	1	....	....	1	1	....	1	....	1	1	+	+	+	+	+	280	44	31	13
24	....	....	1	....	....	1	....	....	....	....	1	1	+	+	+	0	+	240	65	21	44
25	....	....	1	....	....	....	....	....	1	1	....	1	0	+	+	+	+	160	70	52	18
26	....	....	....	....	....	....	1	1	1	1	....	....	0	+	+	+	+	248	3	0	3
27	....	....	1	....	....	....	1	....	....	2	....	1	0	+	+	0	+	272	61	36	25
28	....	....	....	....	....	1	....	....	1	2	....	2	+	+	+	+	+	312	40	0	40
29	....	....	1	1	....	1	....	....	1	1	1	....	+	+	+	+	+	272	74	58	16
30	....	....	1	....	....	....	....	....	....	....	....	....	0	+	+	0	+	312	40	40	0
31	....	....	1	....	....	1	....	....	....	....	1	1	+	+	+	0	+	240	64	22	42
32	....	....	2	....	....	2	....	....	....	....	1	1	+	+	+	0	+	304	57	47	10
33	....	....	....	....	....	....	....	....	....	2	....	....	0	+	+	0	+	160	41	0	41
34	....	....	2	....	....	1	....	....	....	....	....	....	+	+	+	0	+	262	41	41	0
35	....	1	2	....	....	1	....	....	1	....	....	....	+	+	+	+	+	304	55	55	0
36	....	1	....	....	....	1	1	....	....	1	....	....	+	+	+	0	+	352	19	0	19
37	....	....	....	....	....	1	....	1	1	1	....	....	+	+	+	+	+	288	13	0	13
38	1	....	2	....	....	2	....	....	....	....	1	1	+	+	+	0	+	328	66	43	23
39	....	....	....	....	....	1	....	....	1	1	1	1	+	+	+	+	+	192	51	0	51
40	....	1	....	....	....	1	....	....	1	....	1	1	+	+	+	+	+	320	13	0	13
41	....	....	1	....	....	1	....	....	....	....	....	....	+	+	+	0	+	232	18	18	0
42	....	....	1	....	....	1	....	....	....	1	3	....	+	+	+	0	+	240	86	0	86
43	....	....	1	2	1	1	....	....	....	....	....	....	+	+	+	0	+	344	97	97	0
44	....	....	2	....	....	1	1	....	....	....	1	....	+	+	+	0	+	280	76	59	17
45	....	....	1	....	....	1	....	....	....	....	1	1	+	+	+	0	+	336	40	20	20
46	....	....	1	....	....	1	....	....	....	2	1	....	+	+	+	0	+	280	45	0	45
47	....	....	1	....	....	1	....	....	....	....	2	....	+	+	+	0	+	232	47	20	27
48	....	....	1	....	....	1	1	....	1	2	....	....	+	+	+	+	+	264	64	0	64
49	....	....	1	1	....	1	....	....	....	....	....	....	+	+	+	0	+	304	35	35	0
50	....	....	1	....	....	1	1	....	1	1	....	....	+	+	+	+	+	208	67	17	50

Per cent of kernels destroyed by both species	per cent	18.9
Injury attributed to European corn borer (40 per cent of total damage)	do	7.72
Injury attributed to corn earworm (60 per cent of total damage)	do	11.18
Per cent of ears infested:		
By either species	do	100
By both species	do	82
By European corn borer	do	96
By corn earworm	do	86
Average number of corn earworms per ear		1.36
Average number of European corn borers per ear		3.14
Per cent of stalks infested (10 stalks)	per cent	100
Infestation per stalk (excluding ears):		
Greatest	larvæ	23
Least	do	4
Average	do	11.1

CONCLUSIONS

The evidence presented in this paper shows that the corn earworm and the European corn borer may exist in harmony in the same host plants.

In each of the two examinations made 100 per cent of the ears were found infested by one or the other species. Both species were present in 71 per cent of the ears in the first case and in 82 per cent in the second case. The European corn borer infested 88 per cent of the ears in the first instance and 96 per cent in the second. Larvæ of the corn earworm were found in 83 per cent of the ears in the first count and in 86 per cent in the second count.

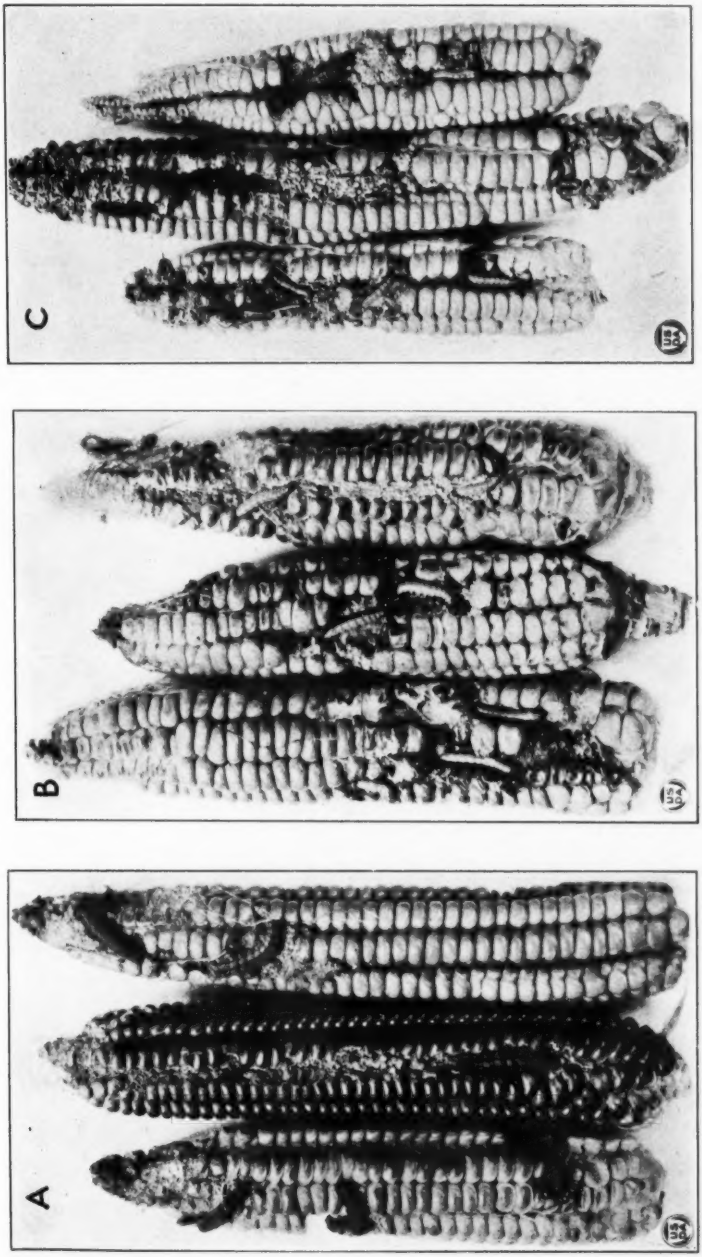
Injury resulting from the feeding of larvæ of these two species was found on October 14, 1921, to be a destruction of 10.5 per cent of the total grain on 100 ears and on October 20, 1921, 18.9 per cent of the total grain on 50 ears. It should be observed at this point that this injury was to fully developed kernels and not to any that had failed to develop because of injury by the European corn borer to other parts of the plants. It was found that on the last-mentioned date the corn earworm was responsible for 60 per cent of the total damage, or 11.18 per cent of actual grain destruction, while 40 per cent of the total damage, or 7.72 per cent of actual grain destruction, was inflicted by the European corn borer. These figures show the total injury caused by the corn earworm, but do not show the whole damage by the European corn borer, such as undeveloped kernels and feeding by the latter species after the corn earworm has become full grown and deserted the ears.

Since it has been shown that these two species may exist in corn, each feeding independently of the other, and each contributing a share to the total injury, it appears that, should the European corn borer ever reach the great corn-producing area in which severe losses occur through feeding of corn earworm larvæ, the total damage resulting from the injury by both species would probably lead to an appreciable decrease in the yield of grain.

PLATE 1

Pyrausta nubilalis and *Heliothis obsoleta*

A.—Ears of Longfellow flint field corn injured by larvæ of *Heliothis obsoleta*.
B.—Ears of Longfellow flint field corn injured by larvæ of *Pyrausta nubilalis*.
C.—Ears of Longfellow flint field corn injured by larvæ of *Heliothis obsoleta* and of *Pyrausta nubilalis*.


C
B
A